ISOLATION OF PHYTOPATHOGENIC FUNGI WITH POTENTIAL AS BIOHERBICIDE FOR THE CONTROL OF INVASIVE WATER HYACINTH, (*Pontederia crassipes)*

Project report submitted to Sister Nivedita University in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

MICROBIOLOGY

Submitted By

MAYUKH PATRA

Registration ID: 230010274452

Enrollment ID: 2321211001027

Advisor

DR. PUJA RAY

Associate Professor

Department of Life Science, Presidency University

School of Health Science and Translational Research

Sister Nivedita University

Kolkata

June 2025

PRESIDENCY UNIVERSITY
KOLKATA

Hindu College (1817-1855), Presidency College (1855-2010)

This is to certify that the dissertation project entitled **'Isolation of phytopathogenic fungi with potential as Bioherbicide for the control of invasive water hyacinth *(Pontederiacrassipes)*"** is carried out by **Mayukh Patra,** Registration No. 230010274452, Enrollment No. 2321211001027 of M.Sc Semester IV, Department of Microbiology, Sister Nivedita University, Newtown, West Bengal - 700156 for the partial fulfilment of the Degree of M.Sc., at the Multitrophic Interactions and Biocontrol Research Laboratory, Department of Life Sciences, Presidency University, Kolkata. The tenure of his work was from January 2025 to May 2025.

The entire dissertation work has been done under my supervision and this work has not been submitted elsewhere for a degree.

Dr. Puja Ray
(Associate Professor)
Department of Life Sciences,
Presidency University,86/1 College Street,
Kolkata-700073, India
Email: puja.dbs@presiuniv.ac.in, Cell phone: +91-9163144757
Research Webpage: https://sites.google.com/view/dr-puja-rays-research-group/homeFaculty profile: http://presiuniv.ac.in/web/staff.php?staffid=35

Dated: 29th May 2025

DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "**Isolation of phytopathogenic fungi with Potential as bioherbicide for the control invasive, Water Hyacinth (*Pontederia crassipes)***" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Microbiology is an authentic record of my own work carried out during the period January, 2025 to May, 2025 under the supervision of Dr. Puja Ray, Department of Life Sciences, Presidency University.

I acknowledge that I have read and understood the UGC regulations, 2018 (*Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions*).

I confirm that this dissertation has been checked for plagiarism using the plagiarism checking software Small SEO Tools. At the end of the dissertation, a copy of the summary report demonstrating similarities in content and its potential source (if any), generated online using plagiarism checking software is enclosed. I herewith confirm that the dissertation has been less than 10% similarity according to the plagiarism checking software's report and meets the UGC Regulations as well as plagiarism rules of Sister Nivedita University.

I further declare that no portion of the dissertation or its data will be published without the Institute's or supervisor's permission. I have not previously applied for any other degree or award using the topics and findings described in my dissertations.

Mayukh Patra
5/6/25

_____

(Signature of the Student with Date)
Name of the Student: MAYUKH PATRA

Registration Number:230010274452

Department of Microbiology, Sister Nivedita University

SISTER NIVEDITA UNIVERSITY
সিস্টার নিবেদিতা বিশ্ববিদ্যালয়

DG Block - 1/2, Action Area I, New Town, Kolkata - 700156, West Bengal, India.

DEPARTMENT OF BIOTECHNOLOGY & DEPARTMENT OF MICROBIOLOGY
জৈব প্রযুক্তি বিভাগ ও অণুজীব বিদ্যা বিভাগ

TO WHOM IT MAY CONCERN

This is to certify that the project report titled " **Isolation of Phytopathogenic Fungi with Potential as bioherbicide for the control invasive, Water Hyacinth (*Pontederia crassipes)*** submitted by Mr. Mayukh Patra (Registration No. 230010274452 ; Enrolment No. 2321211001027) to the Department of Microbiology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a Bonafide record of research conducted by him for the partial fulfillment of the requirements for the Master of Science degree.

Date: 05.06.25

Place: New Town, Kolkata

Signature of Head of the Department

Acknowledgement

I would like to express my deep appreciation to Dr. Puja Ray, Associate Professor in the Department of Life Sciences at Presidency University. Without her invaluable guidance, this project would not have reached a successful completion. Working under her supervision has been an enlightening experience which has helped me gaining knowledge that will undoubtedly benefit me in the future.

I would like to thank Dr. Rajat Pal, Assistant Director, Department of Microbiology and Biotechnology at Sister Nivedita University.

Furthermore, I would like to extend my thanks to Dr. Fatema Calcuttawala, Head of the Department of the Department of Microbiology at Sister Nivedita University.

I am immensely grateful to my Research Scholar Guide Mrs. Payel Basak, Mr. Mayukh Ghosh, Mr. Arnab Sahaandmy laboratory colleagues, Ms. Britisha Naskar and Mr. Niladri Bhowmick. I am also grateful to Ms. Sudeshna Raha and Mr. Kamalendu Sen for their constant help and support. Their guidance, contribution, collaboration and support have been important in the completion of this project.

Additionally, I am thankful to Presidency University for providing me with the necessary resources and facilities that were crucial for a successful completion.

Mayukh Patra

TABLE OF CONTENTS

Serial Number	Topic	Page Number
1.	List of Tables	07
2.	List of Figures	08
3.	Chapter 01: Abstract	17
4.	Chapter 02: Keywords & Abbreviations	17
5.	Chapter 03: Introduction	18
6.	Chapter 04: Review of Literature	19
7.	Chapter 05: Objectives	21
8.	Chapter 06: Materials and Methods 6.1. Isolation 6.1.1. Sample collection 6.1.2. Surface sterilization of collected leaves 6.1.3. Preparation of Potato Dextrose Agar media 6.1.4. Inoculation of diseased leaves 6.1.5. Subculturing to obtain pure culture 6.2. Twig Pathogenicity Test 6.3. Whole Plant Bioassay 6.4. Secondary Metabolite Extraction 6.4.1. Mass culture 6.4.2. Extraction of crude Metabolites 6.4.3. Toxicity Assessment of crude metabolites 6.5. Morphological Identification 6.6.1. Initial identification by staining from plates 6.6.2. Identification by staining from infected leaves 6.6. Statistical Analysis	22
9.	Chapter 07: Results and Discussions 7.1. Inoculation 7.2. Pure culture 7.3. Twig assay 7.4. Whole plant bioassay 7.5. Extraction of crude metabolites 7.6. Toxicity test of metabolites 7.7. Morphological identification 7.7. Statistical analysis	34
10.	Chapter 08: Significance	71
11.	Chapter 09: Future Prospects	72
12.	Chapter 10: Conclusion	73
13.	Chapter 12: References	74
14.	Plagiarism Report	79

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
6.1	List of water hyacinth infested sites along with fungal isolates obtained from diseased leaves of water hyacinth in districts of West Bengal, India from January 2025 to March 2025	22
6.2	Composition of Potato Dextrose Agar (PDB)	23
6.3	Composition of Potato Dextrose Broth (PDB)	28
7.4.1	showing toxicity score on water hyacinth leaves on application of isolated fungus on day 3,5 and 7 respectively,[toxicity score 2(severe damage) followed by disease score 1(mild damage) and disease score 0(no disease or symptoms)].	53
7.5.1	| FD(%) of whole plant bioassay of fungal strains observed on day 3, 5 and 7 respectively.	62
7.5.2	ANOVA table obtained by statistical analysis (SPSS) showing no significant difference between fungal strains on day 3 of whole plant bioassay.	62
7.5.3	ANOVA table obtained by statistical analysis (SPSS) showing no significant difference between fungal strains on day 5 of whole plant bioassay.	63
7.5.4	ANOVA table obtained by statistical analysis (SPSS) showing no significant difference between fungal strains on day 7 of whole plant bioassay.	63
7.7.1	(FD%) of toxicity test of crude metabolites of day 15	69
7.7.2	ANOVA table obtained by statistical analysis (SPSS) showing significant difference between fungal strains	69

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
CHAPTER-03		
3.1	**Water Hyacinth (*Pontederia crassipes*) infested areas observed during field surveys**	**18**
CHAPTER-07		
7.1.1	**Diseased leaves of water hyacinth obtained from various sites**	**34**
7.3.1	**The different fungal strains of water hyacinth were successfully subcultured to obtain this pure culture of fungal strains of water hyacinth**	**35**
7.4	**Toxicity assay of control observed on day 01, 03 and 05 on application with distilled water and T20 solution on A|Normal leaf +D H_2O B| Normal leaf +T20 C|Scratch Leaf + D H_2O D| Scratch Leaf + T20**	**36**
7.4.1	**Toxicity assay of fungus (WH-1) observed on day 01 after application of fungus (WH-1) on the healthy leaves of water hyacinth. A| Fungus(WH1) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-1) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.**	**37**
7.4.2	**Toxicity assay of fungus (WH-1) observed on day 03 after application of fungus (WH-1) on the healthy leaves of water hyacinth. A| Fungus(WH1) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-1) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.**	**37**
7.4.3	**Toxicity assay of fungus (WH-1) observed on day 05 after application of fungus (WH-1) on the healthy leaves of water hyacinth. A| Fungus(WH1) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-1) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.**	**37**
7.4.4	**Toxicity assay of fungus (WH-2) observed on day 01 after application of fungus (WH-2) on the healthy leaves of water hyacinth. A| Fungus(WH-2) with distilled water, B| Fungus(WH-2) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-2) and distilled water, D| On Scratch Leaf with Fungus(WH-2) and Tween20 solution.**	**38**
7.4.6	**Toxicity assay of fungus (WH-2) observed on day 05 after application of fungus (WH-2) on the healthy leaves of water hyacinth. A| Fungus(WH-2) with distilled water, B| Fungus(WH-2) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-2) and distilled water, D| On Scratch Leaf with Fungus(WH-2) and Tween20 solution.**	**38**

7.4.7	**Toxicity assay of fungus (WH-3) observed on day 01 after application of fungus (WH-3) on the healthy leaves of water hyacinth. A| Fungus(WH-3) with distilled water, B| Fungus(WH-3) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-3) and distilled water, D| On Scratch Leaf with Fungus(WH-3) and Tween20 solution**	**39**
7.4.8	**Toxicity assay of fungus (WH-3) observed on day 03 after application of fungus (WH-3) on the healthy leaves of water hyacinth. A| Fungus(WH-3) with distilled water, B| Fungus(WH-3) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-3) and distilled water, D| On Scratch Leaf with Fungus(WH-3) and Tween20 solution.**	**39**
7.4.9	**Toxicity assay of fungus (WH-3) observed on day 05 after application of fungus (WH-3) on the healthy leaves of water hyacinth. A| Fungus(WH-3) with distilled water, B| Fungus(WH-3) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-3) and distilled water, D| On Scratch Leaf with Fungus(WH-3) and Tween20 solution.**	**39**
7.4.10	**Toxicity assay of fungus (WH-4) observed on day 01 after application of fungus (WH-4) on the healthy leaves of water hyacinth. A| Fungus(WH-4) with distilled water, B| Fungus(WH-4) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-4) and distilled water, D| On Scratch Leaf with Fungus(WH-4) and Tween20 solution.**	**40**
7.4.11	**Toxicity assay of fungus (WH-4) observed on day 03 after application of fungus (WH-4) on the healthy leaves of water hyacinth. A| Fungus(WH-4) with distilled water, B| Fungus(WH-4) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-4) and distilled water, D| On Scratch Leaf with Fungus(WH-4) and Tween20 solution.**	**40**
7.4.12	**Toxicity assay of fungus (WH-4) observed on day 05 after application of fungus (WH-4) on the healthy leaves of water hyacinth. A| Fungus(WH-4) with distilled water, B| Fungus(WH-4) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-4) and distilled water, D| On Scratch Leaf with Fungus(WH-4) and Tween20 solution.**	**40**
7.4.13	**Toxicity assay of fungus (WH-5) observed on day 01 after application of fungus (WH-5) on the healthy leaves of water hyacinth. A| Fungus(WH-5) with distilled water, B| Fungus(WH-5) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-5) and distilled water, D| On Scratch Leaf with Fungus(WH-5) and Tween20 solution.**	**41**
7.4.14	**Toxicity assay of fungus (WH-5) observed on day 03 after application of fungus (WH-5) on the healthy leaves of water hyacinth. A| Fungus(WH-5) with distilled water, B| Fungus(WH-5) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-5) and distilled water, D| On Scratch Leaf with Fungus(WH-5) and Tween20 solution.**	**41**
7.4.15	**Toxicity assay of fungus (WH-5) observed on day 05 after application of fungus (WH-5) on the healthy leaves of water hyacinth. A| Fungus(WH-5) with distilled water, B| Fungus(WH-5) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-5) and distilled water, D| On Scratch Leaf with Fungus(WH-5) and Tween20 solution.**	**41**

7.4.16	**Toxicity assay of fungus (WH-6) observed on day 01 after application of fungus (WH-6) on the healthy leaves of water hyacinth. A| Fungus(WH-6) with distilled water, B| Fungus(WH-6) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-6) and distilled water, D| On Scratch Leaf with Fungus(WH-6) and Tween20 solution.**	**42**
7.4.17	**Toxicity assay of fungus (WH-6) observed on day 03 after application of fungus (WH-6) on the healthy leaves of water hyacinth. A| Fungus(WH-6) with distilled water, B| Fungus(WH-6) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-6) and distilled water, D| On Scratch Leaf with Fungus(WH-6) and Tween20 solution.**	**42**
7.4.18	**Toxicity assay of fungus (WH-6) observed on day 05 after application of fungus (WH-6) on the healthy leaves of water hyacinth. A| Fungus(WH-6) with distilled water, B| Fungus(WH-6) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-6) and distilled water, D| On Scratch Leaf with Fungus(WH-6) and Tween20 solution.**	**42**
7.4.19	**Toxicity assay of fungus (WH-7) observed on day 01 after application of fungus (WH-7) on the healthy leaves of water hyacinth. A| Fungus(WH-7) with distilled water, B| Fungus(WH-7) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-7) and distilled water, D| On Scratch Leaf with Fungus(WH-7) and Tween20 solution.**	**43**
7.4.20	**Toxicity assay of fungus (WH-7) observed on day 03 after application of fungus (WH-7) on the healthy leaves of water hyacinth. A| Fungus(WH-7) with distilled water, B| Fungus(WH-7) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-7) and distilled water, D| On Scratch Leaf with Fungus(WH-7) and Tween20 solution.**	**43**
7.4.21	**Toxicity assay of fungus (WH-7) observed on day 05 after application of fungus (WH-7) on the healthy leaves of water hyacinth. A| Fungus(WH-7) with distilled water, B| Fungus(WH-7) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-7) and distilled water, D| On Scratch Leaf with Fungus(WH-7) and Tween20 solution.**	**43**
7.4.22	**Toxicity assay of fungus (WH-8) observed on day 01 after application of fungus (WH-8) on the healthy leaves of water hyacinth. A| Fungus(WH-8) with distilled water, B| Fungus(WH-8) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-8) and distilled water, D| On Scratch Leaf with Fungus(WH-8) and Tween20 solution.**	**44**
7.4.23	**Toxicity assay of fungus (WH-8) observed on day 03 after application of fungus (WH-8) on the healthy leaves of water hyacinth. A| Fungus(WH1) with distilled water, B| Fungus(WH-8) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-8) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.**	**44**
7.4.24	**Toxicity assay of fungus (WH-8) observed on day 05 after application of fungus (WH-8) on the healthy leaves of water hyacinth. A| Fungus(WH-8) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with**	**44**

	Fungus(WH-8) and distilled water, D| On Scratch Leaf with Fungus(WH-8) and 39Tween20 solution.	
7.4.25	**Toxicity assay of fungus (WH-9) observed on day 01 after application of fungus (WH-9) on the healthy leaves of water hyacinth. A| Fungus(WH-9) with distilled water, B| Fungus(WH-9) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-9) and distilled water, D| On Scratch Leaf with Fungus(WH-9) and Tween20 solution.**	**45**
7.4.26	**Toxicity assay of fungus (WH-9) observed on day 03 after application of fungus (WH-9) on the healthy leaves of water hyacinth. A| Fungus(WH-9) with distilled water, B| Fungus(WH-9) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-9) and distilled water, D| On Scratch Leaf with Fungus(WH-9) and Tween20 solution.**	**45**
7.4.27	**Toxicity assay of fungus (WH-9) observed on day 05 after application of fungus (WH-9) on the healthy leaves of water hyacinth. A| Fungus(WH-9) with distilled water, B| Fungus(WH-9) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-9) and distilled water, D| On Scratch Leaf with Fungus(WH-9) and Tween20 solution.**	**45**
7.4.28	**Toxicity assay of fungus (WH-10) observed on day 01 after application of fungus (WH-10) on the healthy leaves of water hyacinth. A| Fungus(WH-10) with distilled water, B| Fungus(WH-10) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-10) and distilled water, D| On Scratch Leaf with Fungus(WH-10) and Tween20 solution**.	**46**
7.4.29	**Toxicity assay of fungus (WH-10) observed on day 03 after application of fungus (WH-10) on the healthy leaves of water hyacinth. A| Fungus(WH-10) with distilled water, B| Fungus(WH-10) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-10) and distilled water, D| On Scratch Leaf with Fungus(WH-10) and Tween20 solution.**	**46**
7.4.30	**Toxicity assay of fungus (WH-10) observed on day 05 after application of fungus (WH-10) on the healthy leaves of water hyacinth. A| Fungus(WH-10) with distilled water, B| Fungus(WH-10) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-10) and distilled water, D| On Scratch Leaf with Fungus(WH-10) and Tween20 solution.**	**46**
7.4.31	**Toxicity assay of fungus (WH-12) observed on day 01 after application of fungus (WH-12) on the healthy leaves of water hyacinth. A| Fungus(WH-12) with distilled water, B| Fungus(WH-12) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-12) and distilled water, D| On Scratch Leaf with Fungus(WH-12) and Tween20 solution.**	**47**
7.4.32	**Toxicity assay of fungus (WH-12) observed on day 03 after application of fungus (WH-12) on the healthy leaves of water hyacinth. A| Fungus(WH-12) with distilled water, B| Fungus(WH-12) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-12) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.**	**47**
7.4.33	**Toxicity assay of fungus (WH-12) observed on day 05 after application of fungus**	**47**

	(WH-12) on the healthy leaves of water hyacinth. A| Fungus(WH-12) with distilled 43water, B| Fungus(WH-12) with Tween20 solution ,C| On Scratch Leaf with Fu43ngus(WH-12) and distilled water, D| On Scratch Leaf with Fungus(WH-12) and 43Tween20 solution.	
7.4.34	**Toxicit44y assay of fungus (WH-13) observed on day 01 after application of fungus (WH-13) 44on the healthy leaves of water hyacinth. A| Fungus(WH-13) with distilled wa44ter, B| Fungus(WH-13) with Tween20 solution ,C| On Scratch Leaf with Fungus(45WH-13) and distilled water, D| On Scratch Leaf with Fungus(WH-13) and Tween2450 solution.**	**48**
7.4.35	**Toxicity assay of f45ungus (WH-13) observed on day 03 after application of fungus (WH-13) on the healthy leaves of water hyacinth. A| Fungus(WH-13) with distilled water, B| Fungus(WH-13) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-13) and distilled water, D| On Scratch Leaf with Fungus(WH-13) and Tween20 solution.**	**48**
7.4.36	**Toxicity assay of fungus (WH-13) observed on day 05 after application of fungus (WH-13) on the healthy leaves of water hyacinth. A| Fungus(WH-13) with distilled water, B| Fungus(WH-13) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-13) and distilled water, D| On Scratch Leaf with Fungus(WH-13) and Tween20 solution.**	**48**
7.4.37	**Toxicity assay of fungus (WH-14) observed on day 01 after application of fungus (WH-14) on the healthy leaves of water hyacinth. A| Fungus(WH-14) with distilled water, B| Fungus(WH-14) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-14) and distilled water, D| On Scratch Leaf with Fungus(WH-14) and Tween20 solution.**	**49**
7.4.38	**Toxicity assay of fungus (WH-14) observed on day 03 after application of fungus (WH-14) on the healthy leaves of water hyacinth. A| Fungus(WH-14) with distilled water, B| Fungus(WH-14) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-14) and distilled water, D| On Scratch Leaf with Fungus(WH-14) and Tween20 solution.**	**49**
7.4.39	**Toxicity assay of fungus (WH-14) observed on day 05 after application of fungus (WH-14) on the healthy leaves of water hyacinth. A| Fungus(WH-14) with distilled water, B| Fungus(WH-14) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-14) and distilled water, D| On Scratch Leaf with Fungus(WH-14) and Tween20 solution**.	**49**
7.4.40	**Toxicity assay of fungus (WH-15) observed on day 01 after application of fungus (WH-15) on the healthy leaves of water hyacinth. A| Fungus(WH1-5) with distilled water, B| Fungus(WH-15) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-15) and distilled water, D| On Scratch Leaf with Fungus(WH-15) and Tween20 solution.**	**50**
7.4.41	**Toxicity assay of fungus (WH-16) observed on day 03 after application of fungus (WH-16) on the healthy leaves of water hyacinth. A| Fungus(WH-16) with distilled water, B| Fungus(WH-16) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-16) and distilled water, D| On Scratch Leaf with Fungus(WH-16) and Tween20 solution.**	**50**

7.4.42	**Toxicity assay of fungus (WH-15) observed on day 05 after application of fungus (WH-15) on the healthy leaves of water hyacinth. A| Fungus(WH-15) with distilled water, B| Fungus(WH-15) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-15) and distilled water, D| On Scratch Leaf with Fungus(WH-15) and Tween20 solution.**	**50**
7.4.43	**Toxicity assay of fungus (WH-18) observed on day 01 after application of fungus (WH-18) on the healthy leaves of water hyacinth. A| Fungus(WH-18) with distilled water, B| Fungus(WH-18) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-18) and distilled water, D| On Scratch Leaf with Fungus(WH-18) and Tween20 solution.**	**51**
7.4.44	**Toxicity assay of fungus (WH-18) observed on day 03 after application of fungus (WH-18) on the healthy leaves of water hyacinth. A| Fungus(WH-18) with distilled water, B| Fungus(WH-18) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-18) and distilled water, D| On Scratch Leaf with Fungus(WH-18) and Tween20 solution.**	**51**
7.4.45	**Toxicity assay of fungus (WH-18) observed on day 05 after application of fungus (WH-18) on the healthy leaves of water hyacinth. A| Fungus(WH-18) with distilled water, B| Fungus(WH-18) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-18) and distilled water, D| On Scratch Leaf with Fungus(WH-18) and Tween20 solution.**	**51**
7.4.46	**Toxicity assay of fungus (WH-19) observed on day 01 after application of fungus (WH-19) on the healthy leaves of water hyacinth. A| Fungus(WH-19) with distilled water, B| Fungus(WH-19) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-19) and distilled water, D| On Scratch Leaf with Fungus(WH-19) and Tween20 solution.**	**52**
7.4.47	**Toxicity assay of fungus (WH-19) observed on day 03 after application of fungus (WH-19) on the healthy leaves of water hyacinth. A| Fungus(WH-19) with distilled water, B| Fungus(WH-19) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-19) and distilled water, D| On Scratch Leaf with Fungus(WH-19) and Tween20 solution.**	**52**
7.4.48	**Toxicity assay of fungus (WH-19) observed on day 05 after application of fungus (WH-19) on the healthy leaves of water hyacinth. A| Fungus(WH-19) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-19) and distilled water, D| On Scratch Leaf with Fungus(WH-19) and Tween20 solution.**	**52**

7.5.2	**Whole plant assay of fungus(WH-1) A| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.**	**54**

7.5.3	Whole plant assay of fungus(WH-2) A| application of fungus (WH-2) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-2) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-2) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.	54
7.5.4	Whole plant assay of fungus(WH-3) A| application of fungus (WH-3) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-3) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-3) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.	55
7.5.5	Whole plant assay of fungus(WH-4) A| application of fungus (WH-4) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-4) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-4) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of) observed on day 05.	55
7.5.6	Whole plant assay of fungus(WH-5) A| application of fungus (WH-5) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-5) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-5) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of) observed on day 05.	56
7.5.7	Whole plant assay of fungus(WH-6) A| application of fungus (WH-6) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-6) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of on day 05 C| application of fungus (WH-6) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.	56
7.5.8	Whole plant assay of fungus(WH-7) A| application of fungus (WH-7) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-7) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-7) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.	57
7.5.9	Whole plant assay of fungus(WH-8) A| application of fungus (WH-8) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B|	57

	application of fungus (WH-8) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-8) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.	
7.5.10	**Whole plant assay of fungus(WH-1) A| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05**	**58**
7.5.11	**Whole plant assay of fungus(WH-10) A| application of fungus (WH-10) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-10) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of Replica1(R1) and Replica2 (R2) observed on day 05 C| application of fungus (WH-10) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.**	**58**
7.5.12	**Whole plant assay of fungus(WH-12) A| application of fungus (WH-12) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-12) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-12) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.**	**59**
7.5.13	**Whole plant assay of fungus(WH-13) A| application of fungus (WH-13) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-13) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-13) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.**	**59**
7.5.14	**Whole plant assay of fungus(WH-14) A| application of fungus (WH-14) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-14) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-14) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.**	**60**
7.5.15	**Whole plant assay of fungus(WH-15) A| application of fungus (WH-15) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-15) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-15) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.**	**60**
7.5.16	**Whole plant assay of fungus(WH-18) A| application of fungus (WH-18) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-18) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-18) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.**	**61**

7.5.17	**Whole plant assay of fungus(WH-19) A| application of fungus (WH-19) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-19) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-19) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.**	**61**
7.5.18	**Effect of different fungal isolates on water hyacinth after 3 days of inoculation Vertical bars represent the standard error of the mean. Means marked with the same letter(s) above the bars(a) are not significantly different (P<0.05).**	**63**
7.5.19	**Effect of different fungal isolates on water hyacinth after 5 days of inoculation Vertical bars represent the standard error of the mean. Means marked with the same letter(s) above the bars(a) are not significantly different (P<0.05).**	**64**
7.5.20	**Effect of different fungal isolates on water hyacinth after 7 days of inoculation Vertical bars represent the standard error of the mean. Means marked with the same letter(s) above the bars(a) are not significantly different (P<0.05).**	**64**
7.6.1	**Fungal metabolites strains of water hyacinth on Potato Dextrose Broth**	**66**
7.7.2	**Application of metabolite spray (WH-2) on the whole plant of water hyacinth observed on day 03, 05 and 07 respectively.**	**67**
7.7.3	**Application of metabolite spray (WH-3) on the whole plant of water hyacinth observed on day 03, 05 and 07 respectively.**	**67**
7.7.4	**Application of metabolite spray (WH-7) on the whole plant of water hyacinth observed on day 03, 05 and 07 respectively.**	**68**
7.7.5	**Application of metabolite spray (WH-9) on the whole plant of water hyacinth of observed on day 03, 05 and 07 respectively.**	**68**
7.7.6	**Application of metabolite spray (WH-19) on the whole plant of water hyacinth observed on day 03, 05 and 07 respectively.**	**68**
7.7.7	**Effect of different fungal isolates on water hyacinth after 15 days of inoculation Vertical bars represent the standard error of the mean. Means marked with the same letter(s) above the bars are not significantly different according to Bonferroni's test (P<0.05).**	**70**
7.8.1	**Morphological characteristics of *Curvularia* species showing typical colony appearances and spore structures under microscope.**	**70**

CHAPTER 01: ABSTRACT

Over the years, the proliferation of aquatic weeds has significantly contributed to the decline of biodiversity within ecosystems. These invasive species often out compete native flora, leading to a reduction in plant diversity and, consequently, the animals that depend on these plants for habitat and food. Phytopathogenic fungi have shown considerable promise as biological control agents against the invasive aquatic weed, water hyacinth (*Pontederia crassipes*).*Alternaria eichhorniae,* for instance, produces phytotoxic compounds like bostrycin and 4-deoxybostrycin, which induce necrotic lesions on the plant's leaves, leading to tissue damage and eventual plant death. Other fungi, such as *Curvularia lunata*, *Fusarium oxysporum*, and *Alternaria alternata*, have been isolated from infected water hyacinth specimens and are known to cause significant tissue-damage, including leaf blight and petiole rot, which compromise the plant's buoyancy and lead to its sinking.The study mainly focused on identifying bioherbicidal agent by isolating phytopathogenic fungus from diseased leaves of water hyacinth from various field surveys around Kolkata followed by pathogenicity test, toxicity test and morphological identification of promising phytopathogenic fungus/fungi.Out of the 16 isolated phytopathogenic fungi,two exhibited highest herbicidal effects, which were morphologically identified as *Curvularia* spp.

CHAPTER 02:KEYWORDS & ABBREVIATIONS

KEYWORDS

Biocontrol, bioherbicides, aquatic weed, invasive

ABBREVIATIONS

FD- Foliar Damage, SE- Standard Error, BOD- Biological Oxygen Demand

CHAPTER 03: INTRODUCTION

Water Hyacinth (*Pontederia crassipes*) is a perennial aquatic plant that floats freely this plant grows quickly, significantly increasing its biomass and creating thick mats, primarily reproducing through stolon's (Gopal et al.,1987). Water hyacinth is an invasive aquatic plant that floats freely and produces flowers, originating from the Amazon Basin in South America. Since 1800s, it has spread from tropical to subtropical regions. In the United States, it has been recognized as an invasive species since 1984(N.R., & Cofrancesco et al., 1989), while in Africa, it has been noted since the early 1900s, in Asia since 1902, and in Europe since the 1930s(Julien et al.,1999). Water hyacinth reproduces both sexually, through seed production via flowers, and asexually, through vegetative budding (Pieterse et al.,1990). This invasive species water hyacinth can double its population in just 5 to 15 days (Penfound et al.,1948). Optimal growth conditions for water hyacinth include nutrient-rich water, temperatures between 28°C and 30°C, a pH range of 6.5 to 8.5, salinity levels below 2%, and nutrient concentrations of 20 mg/L nitrogen, 3 mg/L phosphorus, and 53 mg/L potassium (Reddy et al.,1985).

Figure 3.1| Water Hyacinth (*Pontederia crassipes*) infested areas observed during field surveys

---

Water hyacinth has been labeled as one of the most challenging invasive aquatic species (Gopal et al.,1987) Its swift proliferation can block waterways, diminish biodiversity and the spread of water hyacinth poses significant challenges, including the degradation of water quality, the displacement of native aquatic plants, and the alteration of habitats for fish and other wildlife, and disturb ecological balance (Villamagna et al.,2010; Penfound et al.,1948). Controlling water hyacinth is essential for preserving the integrity of aquatic ecosystems and supporting local economies dependent on un-obstructed waterways. Conventional control strategies, including chemical herbicides and mechanical removal, often prove costly, environmentally damaging, and unsustainable (Julien et al.,1999) (Reddy et al.,1985). Recently, biocontrol agents, especially phytopathogenic fungi, have gained attention as a viable alternative for addressing the proliferation of this invasive species (Charudatta et al.,2001).

CHAPTER 04: REVIEW OF LITERATURE

The aquatic invasive species *Pontederia crassipes*, commonly referred to as water hyacinth, ranks among the most challenging aquatic plants globally (Gopal et al., 1987). Research highlights the critical need to isolate and study fungi from environments heavily infested with water hyacinth to identify those with bioherbicidal potential (Charudattan et al.,2001). The process of isolation typically involves obtaining infected plant samples, applying surface sterilization methods, and cultivating them on specific media that encourage the growth of fungal pathogens (Hoagland et al.,2004). Advances in molecular biology have significantly enhanced the accurate identification of fungal species and the characterization of their virulence factors, shedding light on the mechanisms driving their herbicidal properties. Ultimately, the incorporation of phytopathogenic fungi with bioherbicidal capabilities into strategies for managing water hyacinth offers an environmentally friendly and sustainable solution. (Abdel-Fattah et al.,2001)

1. **Invasiveness of water hyacinth-** *Pontederia crassipes*, commonly known as water hyacinth, is recognized as one of the most invasive aquatic plants worldwide. Originating from South America, it has rapidly spread to tropical and subtropical regions, where it creates significant ecological and economic challenges (Gopal, 1987). The plant's invasiveness is primarily attributed to its high growth rate and ability to reproduce both sexually and vegetatively. Dense mats of water hyacinth block sunlight, disrupt aquatic ecosystems, and reduce oxygen levels in water bodies, leading to the loss of biodiversity (Villamagna & Murphy, 2010). Furthermore, its rapid spread effects water transport, fisheries, and hydropower operations, causing substantial economic losses. The resilience of water hyacinth to varied environmental conditions and its ability to thrive in nutrient-rich waters make it a formidable invasive species.

2. **Conventional Management-** Strategies for controlling water hyacinth include mechanical, manual, and chemical methods. While effective for immediate clearance, this method is costly and labor intensive. Manual removal, though more economical, is time-consuming and often impractical for large infestations (Patel et al., 2012). Chemical control using herbicides such as glyphosate and diquat has shown efficacy in reducing water hyacinth populations. However, the use of chemicals raises concerns regarding environmental pollution, non-target effects, and the potential development of herbicide resistance (Cilliers et al., 2003). Consequently, these methods are often considered short-term solutions that require integration with other strategies.

3. Biological Control- It is a sustainable and environmentally friendly processing managing water hyacinth. The use of natural enemies, such as the weevils *Neochetina eichhorniae* and *Neochetina bruchi*, has been extensively studied and implemented in various countries. These weevils feed on the plant, causing significant reductions in its growth and reproduction (Julien et al., 1999). Other agents, such as the fungal pathogen *Myrothecium roridum*, have also shown promise in controlling water hyacinth. While biological control methods are generally slower than chemical approaches, they offer long-term benefits with minimal environmental impact. However, their success is influenced by factors such as climate, the adaptability of the biocontrol agents, and the extent of the infestation.

4. Phytopathogenic Fungi as Herbicide- The potential of phytopathogenic fungi as bioherbicides for water hyacinth control has gained considerable attention in recent years. Pathogens of water hyacinth such as *Alternaria eichhorniae* and *Fusarium pallidoroseum* have showed efficacy in attacking and suppressing water hyacinth populations (Barreto & Evans, 1996). These fungi invade the plant's tissues, leading to necrosis and death. Unlike chemical herbicides, fungal bioherbicides are environmentally benign and can target the invasive species with high specificity. Research is ongoing to optimize the formulation, delivery, and field application of these pathogens to enhance their effectiveness and scalability.

5. International Approaches- Globally, countries affected by water hyacinth have adopted diverse strategies to combat its spread. In Africa, integrated control programs combining mechanical, chemical, and biological methods have been implemented with varying degrees of success (Hill & Cilliers, 1999). In the United States, biological control using weevils has significantly reduced infestations in several water bodies. International collaborations and knowledge-sharing initiatives, such as those facilitated by the International Organization for Biological Control (IOBC), have played a crucial role in advancing water hyacinth management. These efforts underscore the importance of coordinated approaches that consider ecological, economic, and social dimensions.

6. **National Approaches**- In India, water hyacinth management is a critical component of national aquatic weed control programs. Integrated management practices, including manual removal, herbicide application, and biological control, are employed to tackle infestations in rivers, lakes, and reservoirs. The National Biodiversity Authority (NBA) and state-level agencies work to raise awareness, promote research, and implement control measures. Recent studies have explored the use of indigenous fungal pathogens and other biological agents to develop sustainable and cost-effective solutions (Patel et al., 2019). Community involvement and public-private partnerships are also encouraged to enhance the effectiveness of these initiatives

CHAPTER 05: OBJECTIVES

- Isolation of phytopathogenic fungi from infected water hyacinth leaves.
- Assessment of toxicity on healthy water hyacinth plants.
- Identification of fungi with herbicidal properties against water hyacinth.
- Mass culture of isolated fungi for secondary metabolite productions.

CHAPTER 06: METHODOLOGY

(6.1) ISOLATION

(6.1.1) Sample Collection

Field surveys were conducted in Kolkata and adjacent areas, including North 24 Parganas, South 24 Parganas, Howrah and Birbhum, West Bengal, India, from January 2025 to March, 2025, to mark water hyacinth infested sites and collect diseased diseased water hyacinth leaves. The sites were mapped

SURVEY SITE	LATITUDE(^{0}N)	LONGITUDE(^{0}E)	LABORATORY ISOLATION CODE
Dumdum, North 24 Parganas	22.619762	88.426483	WH-13, WH-14
Dumurjola stadium, Howrah	22.582101	88.306739	WH-1,WH-2,WH-3,WH-9,WH-19
Joka, South 24 Parganas	22.448200	88.301096	WH-6,WH-8
Kudghat, Kolkata	22.473288	88.344472	WH-4,WH-5,WH-18
Kopai, Birbhum	23.712552	87.344472	WH-10, WH-12
Ramrampur,South 24 Parganas	22.272699	88.265591	No isolates Obtained
SatragachiJheel,Howrah	22.583195	88.294239	WH-7, WH-15

Table6.1| List of water hyacinth infested sites along with fungal isolates obtained from diseased leaves of water hyacinth in districts of West Bengal, India from January 2025 to March 2025

Procedure

• Water Hyacinth leaves with visible disease symptoms or signs were collected from the weed-infested sites periodically.

• The collected samples were packaged in layers of dry paper towels (Dutta, Ray, and Ray 2015).

• The water hyacinth samples were transferred into a paper bags and maintained in a cold environment (Souza et al. 2017) by using an ice pack during transportation to the laboratory, where the isolation of fungi was carried out.

(6.1.2) Preparation of Potato Dextrose Agar Media

Potato Dextrose Agar (PDA) is a widely used medium for cultivating fungi, including yeasts and molds. It serves as a general-purpose growth medium and can be enhanced with acids or antibiotics to suppress bacterial contamination. Potato Dextrose Agar (PDA) is utilized in plate count techniques for analyzing food, dairy items, and cosmetic products. Additionally, it supports the growth of medically relevant fungi. The nutrient-rich composition, derived from potato infusion, shows mold sporulation and enhances pigment production in few dermatophytes.

Materials Required

• Erlenmeyer flask

• Sterile Petri plates

• Distilled water

• Potato dextrose agar

• Sterile inoculation loop

• Ethanol 99%

• Spirit lamp

• Autoclave & Laminar air flow cabinet

• Non-absorbent Cotton & permanent marker pen

Composition

Serial Number	Number of constituents	Amount(gm/lt)
01	Potato infusion	200
02	Dextrose	20
03	Agar	15

Table 6.2| Composition of Potato Dextrose Agar (PDB)

Procedure

• All glassware were thoroughly cleaned and dried using hot air oven.

• 1000ml of Potato Dextrose agar media was prepared in an Erlenmeyer flask utilizing the appropriate reagents (Table 6.2).

• Sterilized using a autoclave (at 121°C and 15 psi for 15 minutes) (Pelczar et al.,1993).

• Once the media was sterilized, it was poured in the sterile petri plates and left until it cooled down and solidified with their lid partially open, inside the laminar air flow cabinet (Harrigan et al.,1998).

(6.1.3) Washing and Preparation of Infected Leaves

This step ensures the removal of dust and surface microbial contaminants from infected leaves.

Materials Required

- Distilled Water
- Ethanol (70%)
- Sterile Petri Plate
- Sterile Scalpel and tweezers

Procedure

- Infected leaves were washed under running tap water to remove debris from surface.
- Leaves were immersed in 70% ethanol for 30 seconds to disinfect the surface.(Singleton et al.,2001)
- Leaves were rinsed 2–3 times with distilled water to remove ethanol residues.(Agrios et al.,2005)
- Then the leaves were treated with 0.5% NaOCl solution for 30 seconds for further sterilization.(Harman et al.,1998)
- It was again rinsed with distilled water to remove any remaining sterilant.
- Using sterile scalpel and tweezers, small sections (including necrotic or chlorotic lesions) was excised from the sterilized leaves for inoculation(Campbell et al.,1990).

(6.1.4) Inoculation of Diseased Leaves

This procedure introduces fungal pathogens to diseased leaf tissues for further study.

Materials Required

- Sterile Inoculation Loop
- Spirit Lamp
- Laminar Airflow Cabinet
- Autoclave
- Non-absorbent Cotton
- Permanent Marker Pen

Procedure

1. The inoculation loop was sterilezed by heating in a flame until it glowed red, then dipped in 99% ethanol to cool (Benson et al.,1974).
2. Place a prepared leaf segment on a sterile Petri plate at marked locations(Schaad, et al.,2001).
3. Repeat the sterilization process for each inoculation and ensure no cross-contamination.
4. Limit each Petri plate to a maximum of four inoculated leaf pieces.
5. Plates were placed upside down and incubated at 27°C in a Biological Oxygen Demand (BOD) incubator for a period of 3 to 5 days.(Campbell et al.,1990)

(6.1.5) Subculturing for Pure Cultures

Subculturing involves transferring fungal colonies to fresh growth media to maintain viability and purity.

Materials Required

- Sterile Inoculation Loop
- Spirit Lamp
- Autoclave
- Laminar Airflow Cabinet
- Sterile Distilled Water
- Potato Dextrose Agar (PDA) Medium

Procedure

1. All glassware was cleaned and sterilized in a hot air oven.
2. PDA medium was prepared by mixing media components with distilled water and sterilized at 121°C and 15 psi for 15 minutes (Agrios et al., 2005).
3. The medium was dispensed into Petri plates under aseptic conditions and allowed to solidify.
4. Each plate was labeled with the respective strain's accession number.
5. A small section of the fungal colony was transferred onto the PDA medium using a sterilized inoculation loop (Atlas et al., 2006).
6. Incubation was maintained at 27°C for 3 to 5 days. Afterward, the plates were sealed with parafilm and stored at 4°C for later use (Larone et al., 2011).

(6.2) Toxicity Assay of Water Hyacinth Leaves

This assay evaluates the pathogenic effects of isolated fungi on water hyacinth leaves under controlled conditions.

Materials Required

- Petri Plates
- Tissue Paper
- Absorbent Cotton
- Aluminum Foil
- Sterile Scalpel
- Forceps
- Mortar and Pestle
- Tween-20 Solution

Procedure

- Sanitize and disinfect the beakers using 70% ethanol. Line the beakers with tissue paper and lightly dampen them with tap water *(*Aneja et al., 2004*).*

- Healthy leaves of water hyacinth were selected and was sterilized with 70% ethanol *(*Jiang et al., 2015*).*

- Wrapped the basal portion of each leaf with wet absorbent cotton and enclosed it in aluminium foil *(*Chandramohan & Charudattan et al.,2001*).*

- Prepare a fungal mixture by:

a) Fungal colonies were grinded with distilled water using a mortar and pestle *(*Mishra et al., 2020*),* or
b) Adding Tween-20 solution to fungal colonies and homogenized the mixture *(*Barreto & Evans et al.,1996*).*

- Use sterilized brush to evenly apply the fungal mixture to both sides of the leaves and stems *(*Evans et al., 2000*).*

- Treated leaves were placed into moistened Petri plates using sterile forceps. Seal the plates and maintain moisture *(*Boyette et al., 2008*).*

- Observations were recorded at 24-hour intervals over five days (Day 1, Day 3, and Day 5) *(*Charudattan et al., 2001*).*

(6.3) Whole Plant Assay

Pathogenicity tests were conducted by inoculating seven-day-old fungal cultures onto healthy water hyacinth plants maintained in 500 mL glass beakers. The development of symptoms was observed over a five-day period, with the extent of damage assessed based on discoloration, yellowing, or browning of leaf surfaces *(*Evans et al.,2000*;* Charudattan et al., 2001*).* Damage was categorized into the following: up to 10%, 11–25%, 26–50%, 51–75%, and complete desiccation of the foliar area, including petioles *(*Barreto & Evans et al.,1996*).*

Materials Required

- Glass Beakers (500 mL)
- Healthy Water Hyacinth Plants
- Tissue Paper
- Absorbent Cotton
- Aluminum Foil
- Plastic Wraps
- Tap Water
- Sterile Scalpel
- Sterile Forceps
- Sterile Paint Brush
- Mortar and Pestle

- Distilled Water
- Tween-20 Solution (0.1%)

Procedure

- Glass beakers were thoroughly washed, disinfected with 70% ethanol, and rinsed (Boyette et al., 2008).
- Each beaker was filled with approximately 350 mL of tap water, ensuring the roots of the plants were submerged (Mukherjee et al., 2023).
- Healthy, disease-free water hyacinth plants with intact roots and no feeding marks were selected (Jiang et al., 2015).
- The surfaces of the plants were sterilized using 70% ethanol to eliminate contaminants (Mishra et al., 2020).
- One-square-centimeter sections were cut from agar-grown fungal colonies using a scalpel sterilized with 70% ethanol (Chandramohan & Charudatta et al., 2001).
- The fungal sections were ground into a smooth mixture using a mortar and pestle with the addition of distilled water (Tiwari et al., 2020).
- An additional fungal mixture was prepared by blending with 0.1% Tween-20 solution for enhanced adherence (Barreto & Evans et al., 1996).
- The fungal mixture was evenly distributed across both surfaces of the leaves and stems using a sterilized brush (Boyette et al., 2008).
- The treated plants were placed in beakers containing tap water, ensuring the roots were submerged (Evans et al., 2000).
- The beakers were covered with plastic sheets to maintain optimal humidity for fungal growth (Charudattan, 2001).
- Observations were recorded daily for five days (Day 1, Day 3, and Day 5), with symptoms assessed based on discoloration (yellowing or browning) and damage levels categorized by the percentage of the leaf area affected (Mukherjee et al., 2023).

(6.4)SECONDARY METABOLITES EXTRACTED FROM PHYTOPATHOGENIC FUNGUS WITH HERBICIDAL PROPERTIES AGAINST WATER HYACINTH

(6.4.1) Mass Culture of Isolated Fungus

Producing sufficient quantities of secondary metabolites requires the mass cultivation of fungal isolates under optimized conditions. It refers to the large-scale cultivation of a fungal species (Zhao et al., 2019; Cairns et al., 2018) that has been isolated and identified, usually to produce biomass, secondary metabolites, enzymes, or other bioactive compounds for various purposes. It involves scaling up the growth of a specific fungal strain from a small, isolated sample into larger quantities under controlled conditions. The primary goal is to produce biomass or specific metabolites of interest, such as enzymes, antibiotics, organic acids, or secondary metabolites (Singh et al., 2021). This process requires meticulous planning and control to ensure optimal growth and product yield.enabling

the large-scale production of biologically significant compounds (Pandey et al., 2000). Advances in bioprocess engineering, molecular biology, and environmental control continue to enhance the efficiency and application of fungal mass culture in diverse fields (Meyer et al., 2016). It has wide applications in production of enzymes (e.g., cellulase, amylase, protease) Fermentation products such as citric acid and ethanol, Biodegradation and bioremediation efforts.

Materials Required

- Erlenmeyer flask
- Cotton
- Sterile inoculation loop
- Bunsen burner
- Tissue paper
- Measuring cylinder
- Ethanol (70%)
- Potato dextrose Broth
- Distilled water
- Fungal culture (example from PDA or slant)
- Autoclave
- Orbital shaker (for aeration)

Composition of potato dextrose broth

Constituents	Amount (gm/lt)
Potato infusion	200 gm/lt
Dextrose	20 gm/lt

Table 6.3| Composition of Potato Dextrose Broth (PDB)

PROCEDURE

A) Preparation of Potato Dextrose Broth (PDB)

- A cleaned and washed 1-liter Erlenmeyer flask was selected for use *(*Kumar et al., 2018).
- One litre of distilled water was measured using a measuring cylinder and poured into the flask *(*Patil et al., 2017).
- 24 g of Potato Dextrose Broth (PDB) powder was weighed using a digital weighing machine and added to the flask containing distilled water *(*Brown et al., 2010*).*
- The mixture was stirred using a magnetic stirrer until it was uniformly dissolved *(*Smith & Jones et al., 2015*).*
- The flask was cotton-plugged and autoclaved at 121 psi for 15 minutes *(*Reddy et al., 2021*).*

- After autoclaving, the PDB was allowed to cool inside a laminar airflow cabinet to prevent contamination *(*Gupta & Sharma et al., 2019*).*
- Once cooled, the PDB was distributed equally into 100 mL Erlenmeyer flasks, cotton-plugged, and maintained under aseptic conditions within the laminar airflow cabinet *(*Chandramohan & Charudattan et al.,2001*).*

B) Inoculation of Fungal Strains on Potato Dextrose Broth

- Different fungal strains of water hyacinth (WH-1, WH-2, WH-3, WH-4, WH-5, WH-6, WH-7, WH-8, WH-9, WH-10, WH-12, WH-13, WH-14, WH-15, WH-18, WH-19) were inoculated into the prepared PDB.
- Sterile inoculation loops were used to transfer fungal strains under aseptic conditions within the laminar airflow cabinet *(*Mishra et al., 2020*).*
- A loopful of fungal culture was introduced into labelled 100 mL PDB flasks corresponding to each strain. The contents were gently mixed, and the flasks were resealed with cotton plugs *(*Patil et al., 2017*).*
- Three replicates were prepared for each fungal strain to ensure reproducibility *(*Evans et al., 2000*).*
- The inoculated flasks were incubated at 27°C for 21 days, maintaining uniform growth conditions across all samples *(*Charudattan et al.,2001*).*

(6.4.2) Extraction crude metabolites

The extraction of crude metabolites is a fundamental step in the study of natural products and bioactive compounds derived from biological sources such as plants, microorganisms, and animals (Harvey et al., 2015). This process involves isolating the metabolite-rich extracts from the raw biological material, which can then be further analyzed or used in downstream applications. Crude metabolite extraction is critical in fields such as pharmaceuticals, biotechnology, and agriculture for drug discovery, bioassay development, and understanding metabolic pathways (Eloff, 1998). Certain applications include Drug Discovery: Initial crude extract screening for therapeutic potential(Newman & Cragg, 2020). Bioassays: Testing antimicrobial, antioxidant, or anticancer activity. Metabolites Studies: Profiling the metabolite of biological samples.

By extracting these metabolites in their crude form researchers can screen them for biological activity before investing in time consuming and expensive purification process. Crude extracts also help preserve natural balance of compounds as they exist in the organism which can sometimes lead to stronger or synergistic effects in bioassays (Harvey et al., 2015). Moreover, crude extraction allows scientists to quickly analyze chemical profile of an organism and determine whether it is promising for further study. The resulting crude extract contains wide variety of compounds that can later be separated and identified through purification and analytical techniques (Schmid et al., 2017).

MATERIALS REQUIRED

- Funnel
- Centrifuge system
- Falcons
- Whatman paper
- Erlenmeyers flasks
- Ethanol (70%)
- Cotton
- Label and marker
- Tissue paper

PROCEDURE

- After 21 days of incubation, fungal cultures were removed from the growth chamber *(*Boyette et al., 2008*).*
- Equal portions of the cultures were transferred into sterilized Falcon tubes labeled with strain names and replicate numbers *(*Gupta & Sharma et al., 2019*).*
- The Falcon tubes were centrifuged at 6000 rpm for 15 minutes to separate fungal cells from the culture medium (*Chandramohan & Charudattan et al., 2001*).
- The supernatant was collected using a sterilized funnel lined with Whatman filter paper and transferred to sterilized Erlenmeyer flasks *(*Reddy et al., 2021*).*
- This process was repeated for all fungal strains to obtain cell-free filtrates for subsequent analysis,

(6.4.3) Toxicity test for extracted metabolites

Toxicity testing is a cornerstone in evaluating the safety and potential risks associated with bioactive metabolites, whether derived from natural sources or synthetically produced (Ekwall, 1999). These tests are crucial in ensuring that bioactive compounds are safe for intended applications, including pharmaceuticals, agriculture, and food products. By assessing the potential adverse effects on biological systems, toxicity tests provide essential data to determine safe dosage levels and identify possible toxicological risks (Baldrick, 2005).

MATERIALS REQUIRED

- Healthy Whole plant of water hyacinth
- Tap water
- Glass beakers
- Plastic covers
- Spray bottles
- Ethanol (70%)
- Clean towel
- Distilled water

PROCEDURE

- Healthy water hyacinth plants were placed in sterilized beakers filled with tap water, ensuring that their roots remained submerged. Multiple replicates were created for each test *(*Boyette et al., 2008*).*
- Spray bottles were sterilized using ethanol and rinsed with distilled water. The bottles were labeled according to the fungal strain used *(*Mishra et al., 2020*).*
- The extracted fungal metabolites were poured into the sterilized spray bottles under aseptic conditions *(*Chandramohan & Charudattan et al.,2001*).*
- The aerial parts of the plants were sprayed with the metabolites until thoroughly coated. Excess drips were wiped from the beakers using a clean, sterilized towel *(*Gupta & Sharma et al., 2019*).*
- The beakers were covered with plastic wraps to maintain optimal humidity for the treatment *(*Evans et al., 2000*).*
- Metabolites were reapplied at intervals of five days (Day 5, Day 10, Day 15). Observations were recorded at each interval to monitor changes in plant health and symptoms *(*Mukherjee et al., 2023*).*

(6.4.4): STATISTICAL ANALYSIS

To assess how fungal pathogens and their metabolites affect water hyacinth (*Pontederia crassipes*), a systematic statistical approach is followed. Data from experiments, such as measurements of weed biomass reduction, chlorophyll degradation, and mortality rates—are analysed using analysis of variance (ANOVA). This method determines whether there are significant differences between various treatment groups in the study (Gomez & Gomez, 1984). Once significant effects are identified post-hoc tests are applied. These comparisons help identify which treatments, such as specific fungal isolates or metabolite doses, produce the most pronounced effects on the target weed (Zar, 1999).

All analyses are carried out using statistical software SPSS, which ensures precision, consistency, and replicability. This statistical workflow is essential for understanding the effectiveness of fungal-based biocontrol agents and their potential use in environmentally friendly weed management practices.

6.4.5 Morphological Identification of the Infecting Fungal Species

Fungi associated with plant diseases present a wide range of morphological traits, making morphological analysis a crucial tool for correct identification. This study aims to detect and define the morphological characteristics of fungal spores to facilitate taxonomy classification. By using the slide cellophane technique (Butler and Mann et al.,1959), fungal isolates isolated from water hyacinth leaves that were showing signs and symptoms were subjected to a simple staining method. Spore-producing structures can be clearly observed in their native configuration using this technique. A Zeiss Axioscope A1 microscope was used for the analysis, which allowed for high-resolution viewing of fungal reproductive structures for genus-level identification. The isolates were characterized based on several morphological parameters, including colony growth patterns and the structure and abundance of sporulation. These features were carefully compared with descriptions available in standard mycological references (Gilman et al., 1959; Barnett et al., 1960) (Ellis et al., 1976, 1971) to ensure accurate identification. This morphological approach provides valuable insights into the diversity of fungal species associated with alligator weed and contributes to a better understanding of their taxonomy and potential pathogenicity.

Initial Morphological Identification of isolated Fungal strains directly from the fungal plates

Reagents Required

Lactophenol cotton blue stain

Materials required

- Grease free microscopic glass slides
- Cellophane tape
- Cover slip
- Tissue Paper
- Microscope

Procedure

- A pure culture plate containing the fungal strain was securely sealed and incubated at 28°C for 15 days (Pitt & Hocking et al.,2009).
- A clean glass slide was placed on the work surface, and a drop of Lactophenol cotton blue stain was added (Alexopoulos et al., 1996).
- A small piece of cellophane tape was cut and gently pressed onto the fungal colony. The sticky tape was then positioned over the stain on the slide (Samson et al., 2010).
- Folded tissue paper was used to gently rub the slide, removing any excess stain (Barnett & Hunter et al., 1998).
- The slide was allowed to air dry and observed under a microscope at 40X magnification (De Hoog et al., 2000).

Staining of the infecting fungus from infected leaves

Reagents Required

Lactophenol cotton blue stain

Materials required

- Grease free microscopic glass slides
- Cellophane tape
- Cover slip
- Tissue Paper
- Microscope

Procedure

- Erlenmeyer flasks were thoroughly cleaned, disinfected with 70% ethanol, and filled with tap water (Pitt & Hocking et al., 2009).
- Healthy water hyacinth plants were washed with tap water and then surface-sterilized by immersion in 70% ethanol for 30 seconds, followed by distilled water rinsing (Alexopoulos et al., 1996).
- The shoots were acclimatized in the laboratory environment for five days, with the beakers covered with plastic wraps to maintain a moist atmosphere (Barnett & Hunter et al., 1998).
- A scalpel was sterilized with 70% ethanol, and one-square-centimeter pieces of fungal colonies were carefully excised from the agar plate (Samson et al., 2010).
- The mortar and pestle were wiped with 70% ethanol, and fungal colonies were mixed with a few drops of Tween-20 solution. The mixture was ground to a fine consistency (De Hoog et al., 2000).
- A sterilized brush was used to evenly apply the fungal mixture to the leaves and stems of the water hyacinth (Barreto & Evansey et al., 1996).
- The inoculated shoots were placed in water-filled Erlenmeyer flasks using sterilized forceps, and the flasks were covered with plastic wraps to maintain humidity (Charudattan et al., 2001).
- The plants were grown in a plant growth culture rack with controlled lighting for 5–7 days (Gomez & Gomez et al., 1984).
- A leaf showing necrotic or chlorotic symptoms was removed from the inoculated shoots. A drop of Lactophenol cotton blue stain was applied to the leaf (Alexopoulos et al., 1996).
- The leaf was placed on a slide, covered with a cover slip, and observed under a microscope for fungal structures (Barnett & Hunter et al., 1998).

CHAPTER 07: RESULTS AND DISCUSSIONS

7.1 Disease Survey and Isolation of Pathogenic Fungi

Samples of diseases water hyacinth leaves were collected during the field surveys in Kolkata and adjacent areas, including North 24 Parganas, South 24 Parganas, Howrah and Birbhum, West Bengal, India, from January 2025 to March, 2025. Most of the diseased water hyacinth leaves showed leaf spots and chlorotic and necrotic lesionson the surface of the leaves . Sixteen fungal isolates,(WH-1,WH-2,WH-3, WH-4, WH-5, WH-6, WH-7, WH-8,WH-9,WH-10,WH-12,WH-13, WH-14,WH-15,WH-18,WH-19)lab accession numbers were successfully isolated and purified from the diseased water hyacinth leaves from the above- stated survey sites.

Figure 7.1.1| Diseased leaves of water hyacinth obtained from various sites

---

7.2 Inoculation of diseased leaves on Potato Dextrose agar

The inoculated plates of diseased leaves of water hyacinth in Potato Dextrose Agar showed different fungal colonies on each of the following inoculated plates, that was observed after 3-5 days after the incubated period .

7.3 Sublcultured to obtain pure culture

Obtained pure culture, derived from sub-culturing a single colony containing only one type of microorganisms making it ideal for studies , accurate identification ,characteristics and their behavior .

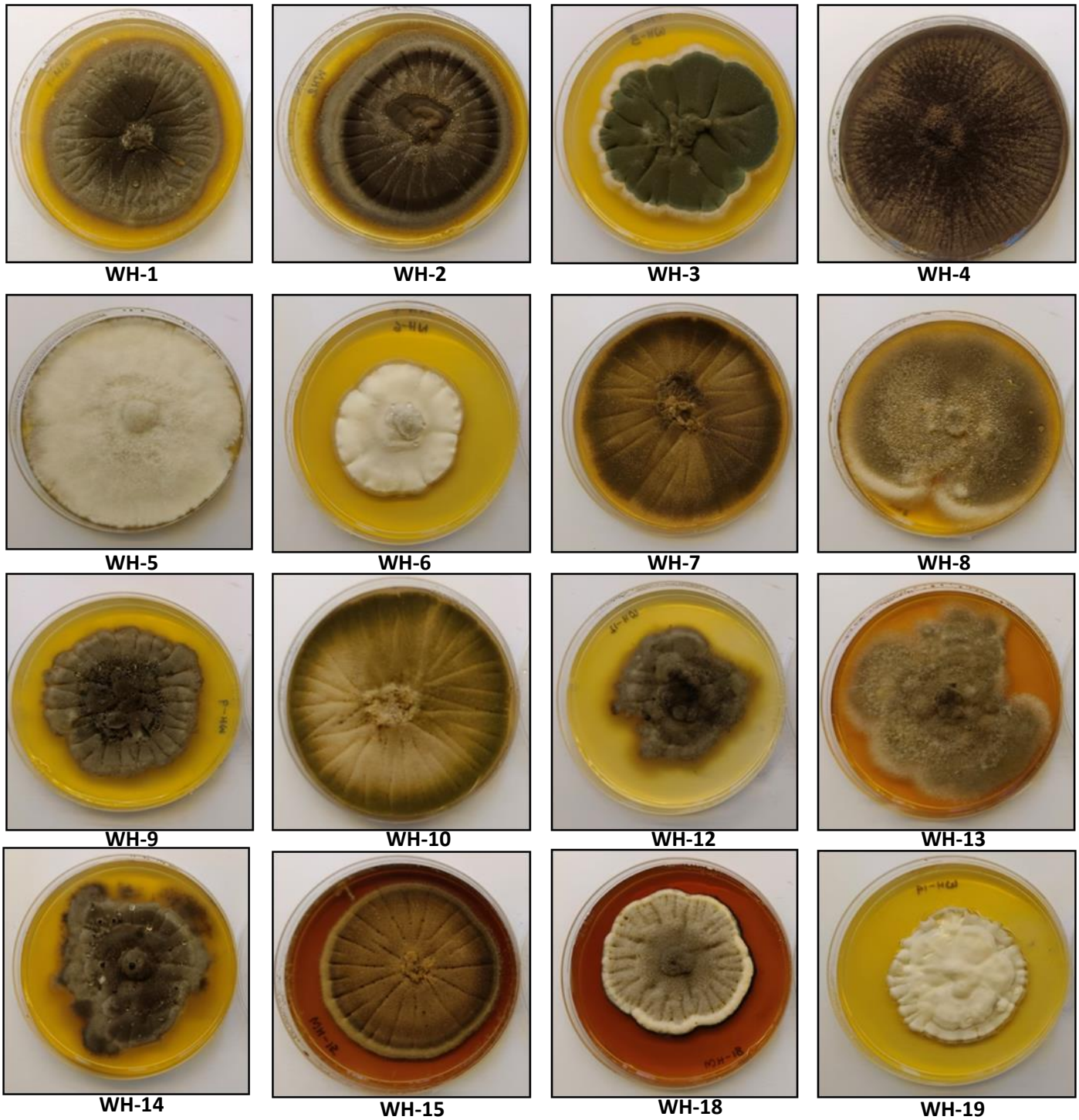
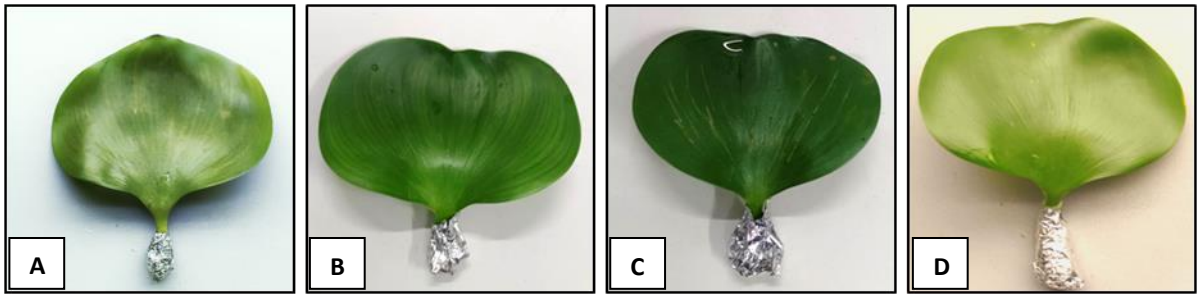


Figure7.3.1 |The different fungal strains i.e., (WH-1,WH-2,WH-3, WH-4, WH-5, WH-6, WH-7, WH-8,WH-9,WH-10,WH-12,WH-13, WH-14,WH-15,WH-18,WH-19) lab accession numbers were successfully subcultured to obtain this pure culture of fungal strains of water hyacinth

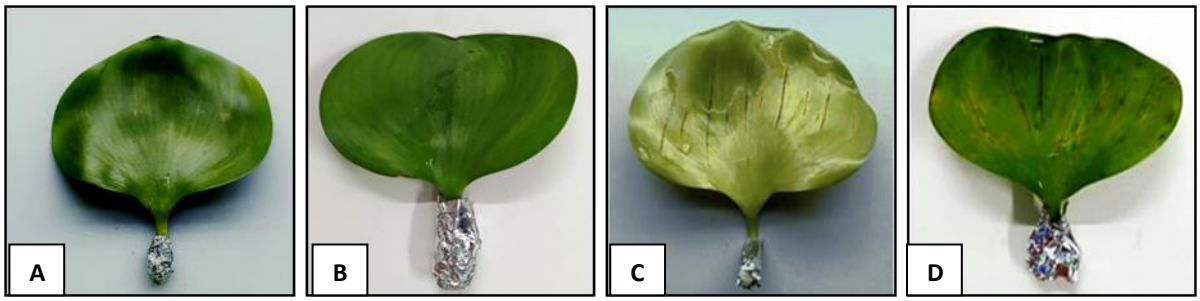
7.4 Toxicity Assay of Water Hyacinth leaves

Among 16 isolates, all the sixteen(WH-1,WH-2,WH-3, WH-4, WH-5, WH-6, WH-7, WH-8,WH-9,WH-10,WH-12,WH-13, WH-14,WH-15,WH-18,WH-19) caused severe leaf damage, within 5 days of application,with disease score 2(severe damage) followed by disease score 1(mild damage) and disease score 0(no disease or symptoms).While the control showed negligible disease or symptoms. An the sixteen isolates showing promising results in toxicity assay were selected for whole plant assay.

CONTROL [DAY-01]



CONTROL [DAY-03]



CONTROL [DAY-05]

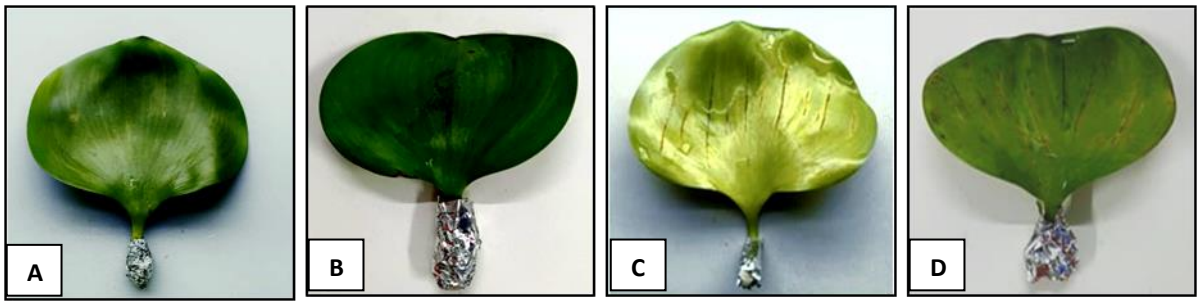
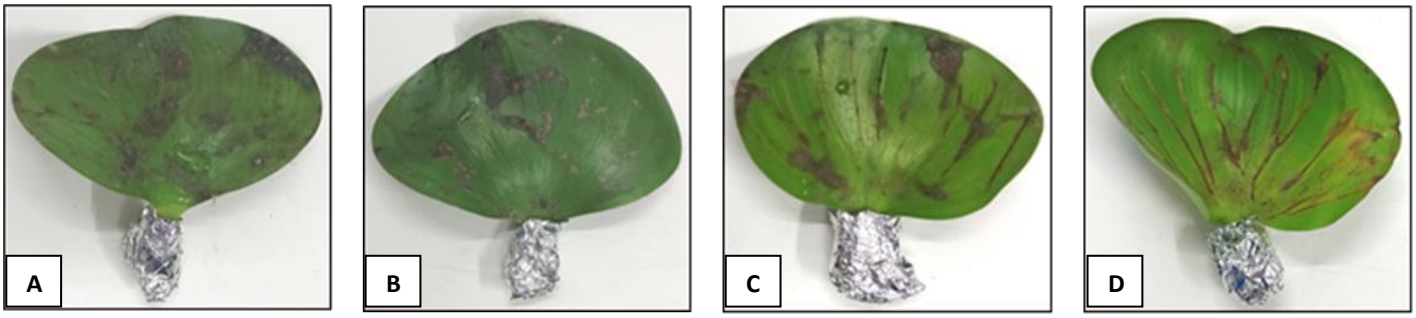


Figure7.4 |Toxicity assay of control observed on day 01, 03 and 05 on application with distilled water and T20 solution on A|Normal leaf +D H_2O B| Normal leaf +T20 C|Scratch Leaf + D H_2O D| Scratch Leaf + T20

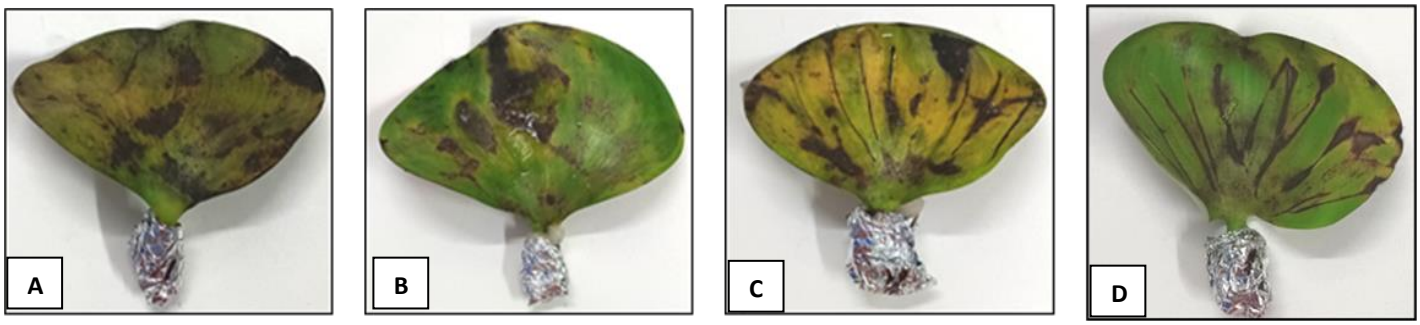
WH-1 [DAY-01]



Fungus(WH-1) + d.H2O | Fungus(WH-1) + T.20 | Scratch Leaf+ Fungus (WH-1) + d.H2O | Scratch Leaf+ Fungus (WH-1) + T.20

Figure7.4.1 | Toxicity assay of fungus (WH-1) observed on day 01 after application of fungus (WH-1) on the healthy leaves of water hyacinth. A| Fungus(WH1) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-1) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.

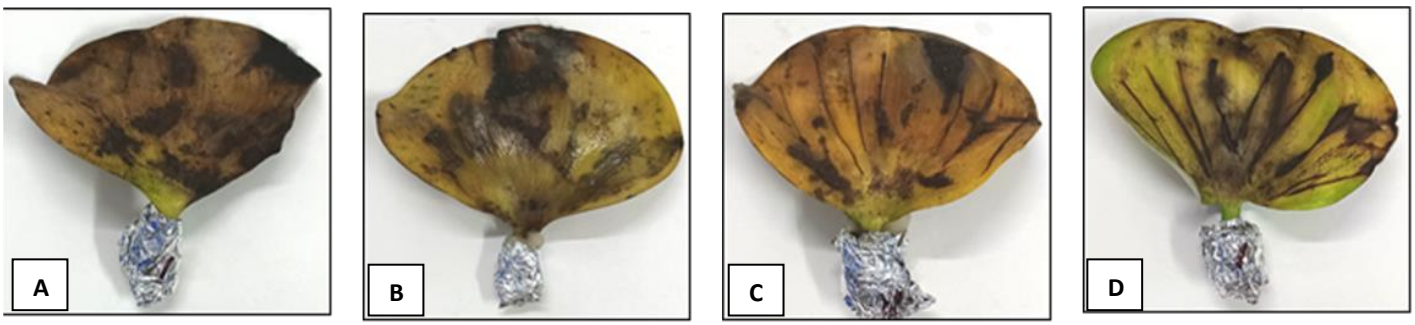
WH-1 [DAY-03]



Fungus(WH-1) + d.H2O | Fungus(WH-1) + T.20 | Scratch Leaf+ Fungus (WH-1) + d.H2O | Scratch Leaf+ Fungus (WH-1) + T.20

Figure7.4.2| Toxicity assay of fungus (WH-1) observed on day 03 after application of fungus (WH-1) on the healthy leaves of water hyacinth. A| Fungus(WH1) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-1) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.

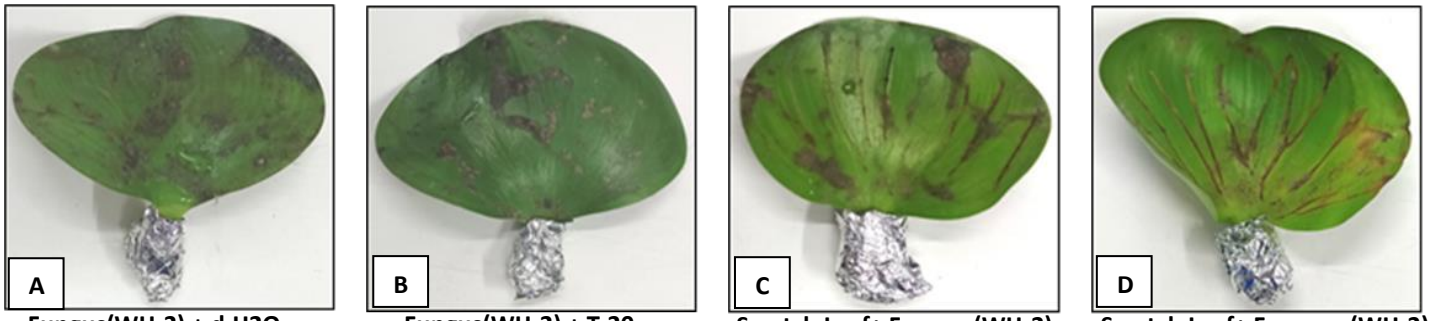
WH-1 [DAY-05]



Fungus(WH-1) + d.H2O | Fungus(WH-1) + T.20 | Scratch Leaf+ Fungus (WH-1) + d.H2O | Scratch Leaf+ Fungus (WH-1) + T.20

Figure7.4.3 | Toxicity assay of fungus (WH-1) observed on day 05 after application of fungus (WH-1) on the healthy leaves of water hyacinth. A| Fungus(WH1) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| OnScratch Leaf with Fungus(WH-1) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.

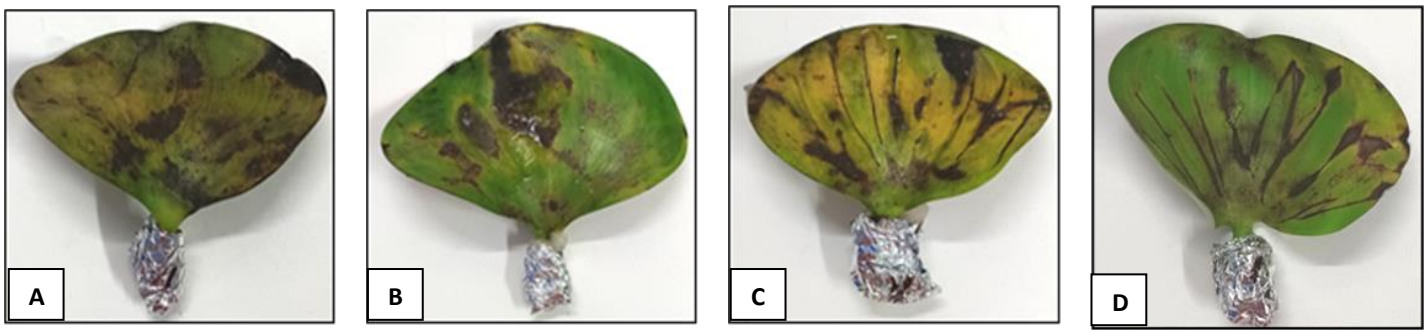
WH-2 [DAY-01]



Fungus(WH-2) + d.H2O | Fungus(WH-2) + T.20 | Scratch Leaf+ Fungus (WH-2) + d.H2O | Scratch Leaf+ Fungus (WH-2) + T.20

Figure7.4.4 | Toxicity assay of fungus (WH-2) observed on day 01 after application of fungus (WH-2) on the healthy leaves of water hyacinth. A| Fungus(WH-2) with distilled water, B| Fungus(WH-2) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-2) and distilled water, D| On Scratch Leaf with Fungus(WH-2) and Tween20 solution.

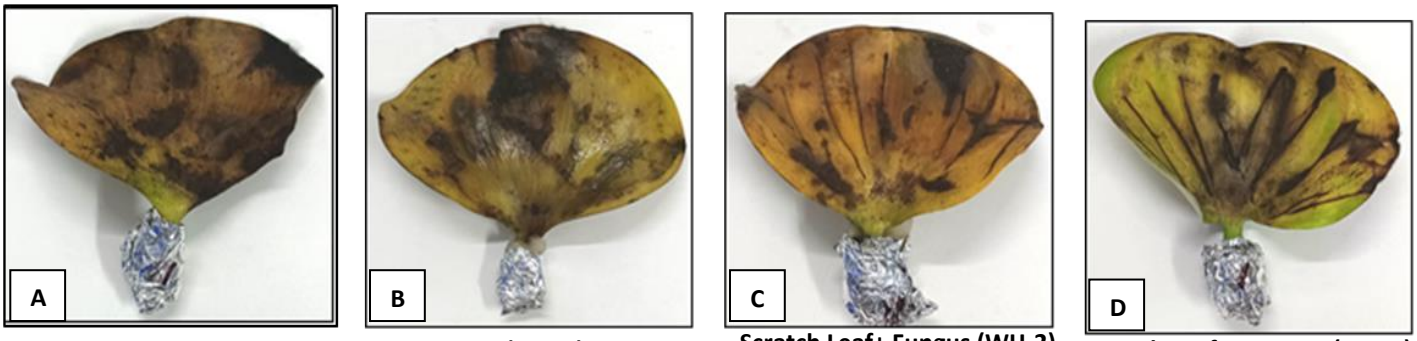
WH-2 [DAY-03]



Fungus(WH-2) + d.H2O | Fungus(WH-2) + T.20 | Scratch Leaf+ Fungus (WH-2) + d.H2O | Scratch Leaf+ Fungus (WH-2) + T.20

Figure7.4.5 | Toxicity assay of fungus (WH-2) observed on day 03 after application of fungus (WH-2) on the healthy leaves of water hyacinth. A| Fungus(WH-2) with distilled water, B| Fungus(WH-2) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-2) and distilled water, D| On Scratch Leaf with Fungus(WH-2) and Tween20 solution.

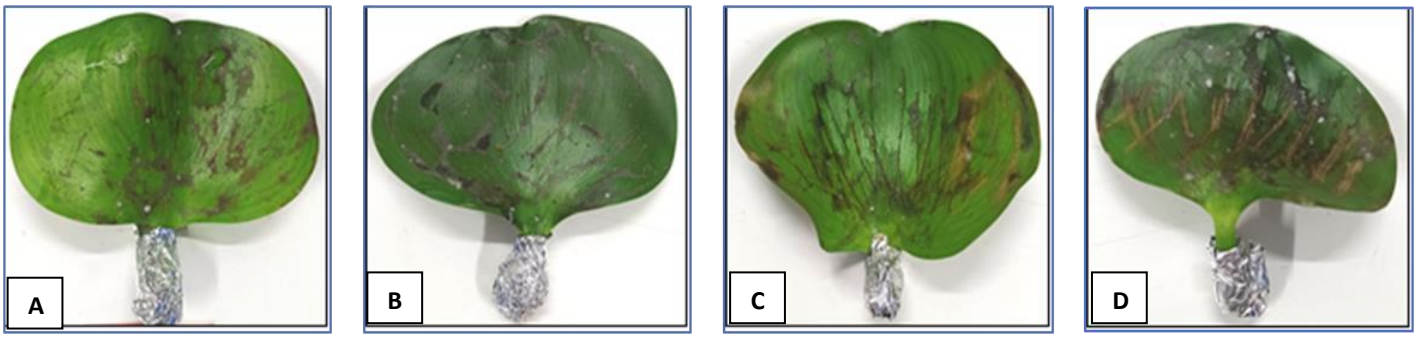
WH-2 [DAY-05]



Fungus(WH-2) + d.H2O | Fungus(WH-2) + T.20 | Scratch Leaf+ Fungus (WH-2) + d.H2O | Scratch Leaf+ Fungus (WH-2) + T.20

Figure7.4.6 | Toxicity assay of fungus (WH-2) observed on day 05 after application of fungus (WH-2) on the healthy leaves of water hyacinth. A| Fungus(WH-2) with distilled water, B| Fungus(WH-2) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-2) and distilled water, D| On Scratch Leaf with Fungus(WH-2) and Tween20 solution.

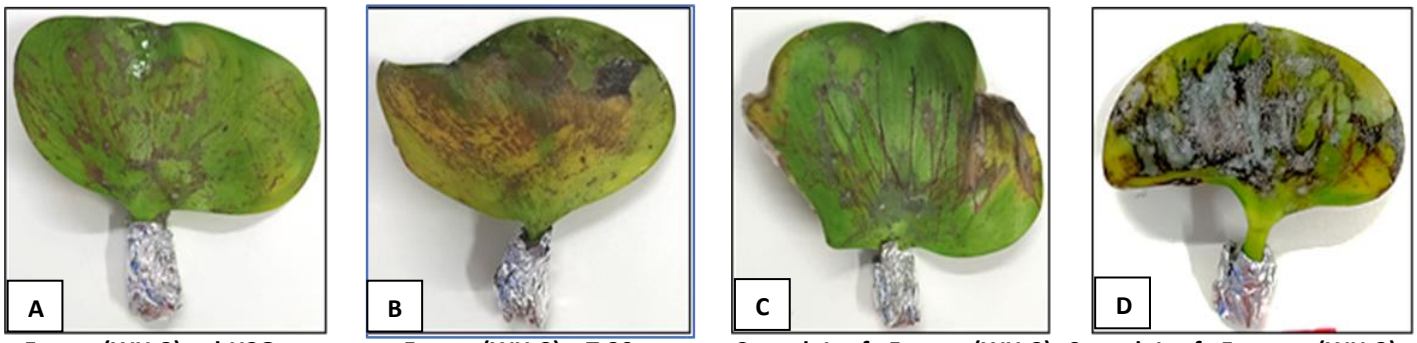
WH-3 [DAY-01]



Fungus(WH-3) + d.H2O | Fungus(WH-3) + T.20 | Scratch Leaf+ Fungus (WH-3) + d.H2O | Scratch Leaf+ Fungus(WH-3) + T.20

Figure7.4.7 | Toxicity assay of fungus (WH-3) observed on day 01 after application of fungus (WH-3) on the healthy leaves of water hyacinth. A| Fungus(WH-3) with distilled water, B| Fungus(WH-3) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-3) and distilled water, D| On Scratch Leaf with Fungus(WH-3) and Tween20 solution.

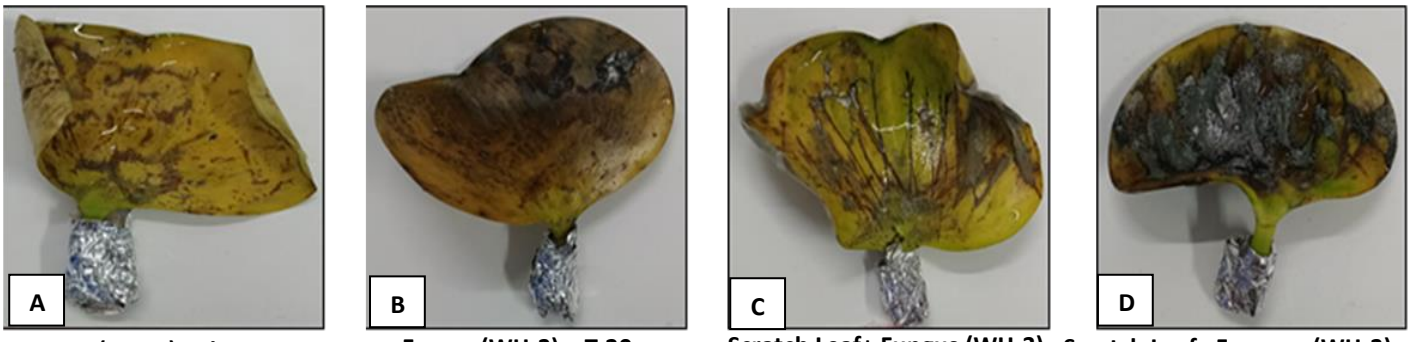
WH-3 [DAY-03]



Fungus(WH-3) + d.H2O | Fungus(WH-3) + T.20 | Scratch Leaf+ Fungus (WH-3) + d.H2O | Scratch Leaf+ Fungus (WH-3) + T.20

Figure7.4.8 | Toxicity assay of fungus (WH-3) observed on day 03 after application of fungus (WH-3) on the healthy leaves of water hyacinth. A| Fungus(WH-3) with distilled water, B| Fungus(WH-3) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-3) and distilled water, D| On Scratch Leaf with Fungus(WH-3) and Tween20 solution.

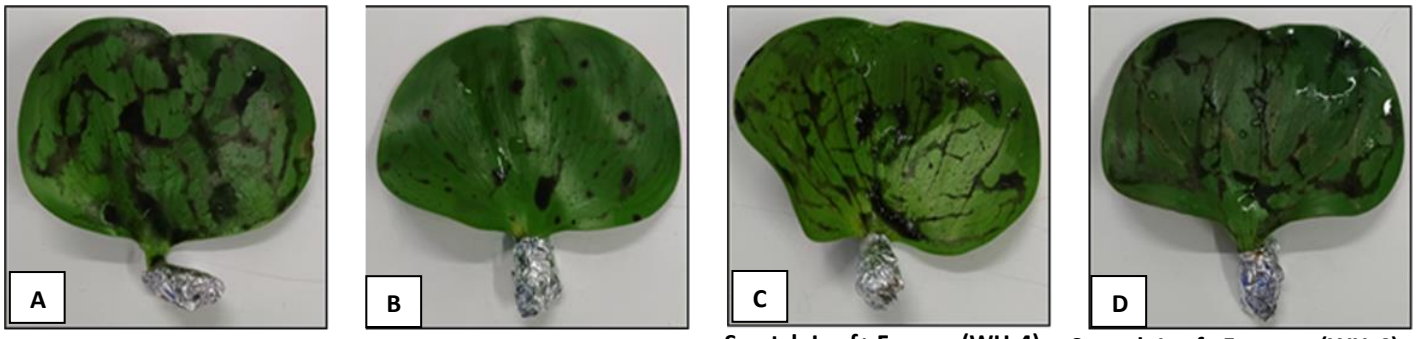
WH-3 [DAY-05]



Fungus(WH-3) + d.H2O | Fungus(WH-3) + T.20 | Scratch Leaf+ Fungus (WH-3) + d.H2O | Scratch Leaf+ Fungus (WH-3) + T.20

Figure7.4.9 | Toxicity assay of fungus (WH-3) observed on day 05 after application of fungus (WH-3) on the healthy leaves of water hyacinth. A| Fungus(WH-3) with distilled water, B| Fungus(WH-3) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-3) and distilled water, D| On Scratch Leaf with Fungus(WH-3) and Tween20 solution.

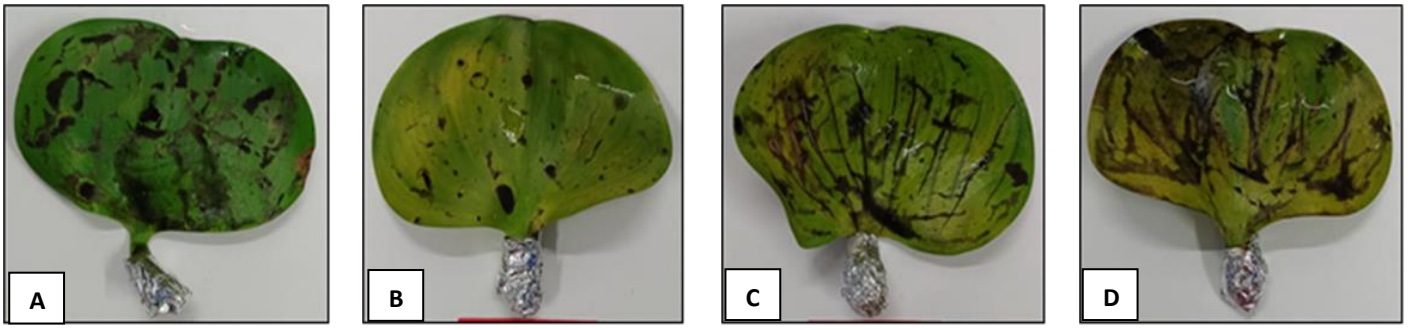
WH-4 [DAY-01]



Fungus(WH-4) + d.H2O | Fungus(WH-4) + T.20 | Scratch Leaf+ Fungus (WH-4) + d.H2O | Scratch Leaf+ Fungus (WH-4) + T.20

Figure7.4.10 | Toxicity assay of fungus (WH-4) observed on day 01 after application of fungus (WH-4) on the healthy leaves of water hyacinth. A| Fungus(WH-4) with distilled water, B| Fungus(WH-4) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-4) and distilled water, D| On Scratch Leaf with Fungus(WH-4) and Tween20 solution.

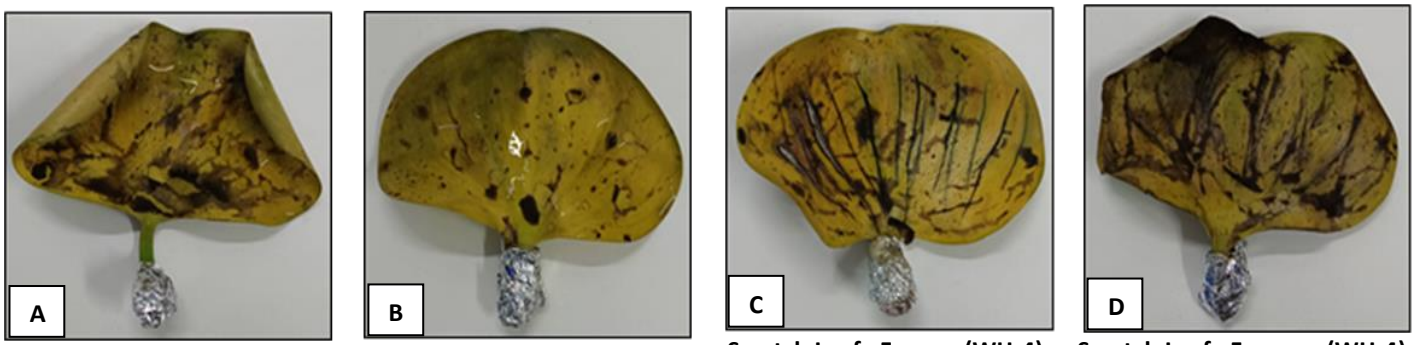
WH-4 [DAY-03]



Fungus(WH-4) + d.H2O | Fungus(WH-4) + T.20 | Scratch Leaf+ Fungus (WH-4) + d.H2O | Scratch Leaf+ Fungus (WH-4) + T.20

Figure7.4.11 | Toxicity assay of fungus (WH-4) observed on day 03 after application of fungus (WH-4) on the healthy leaves of water hyacinth. A| Fungus(WH-4) with distilled water, B| Fungus(WH-4) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-4) and distilled water, D| On Scratch Leaf with Fungus(WH-4) and Tween20 solution.

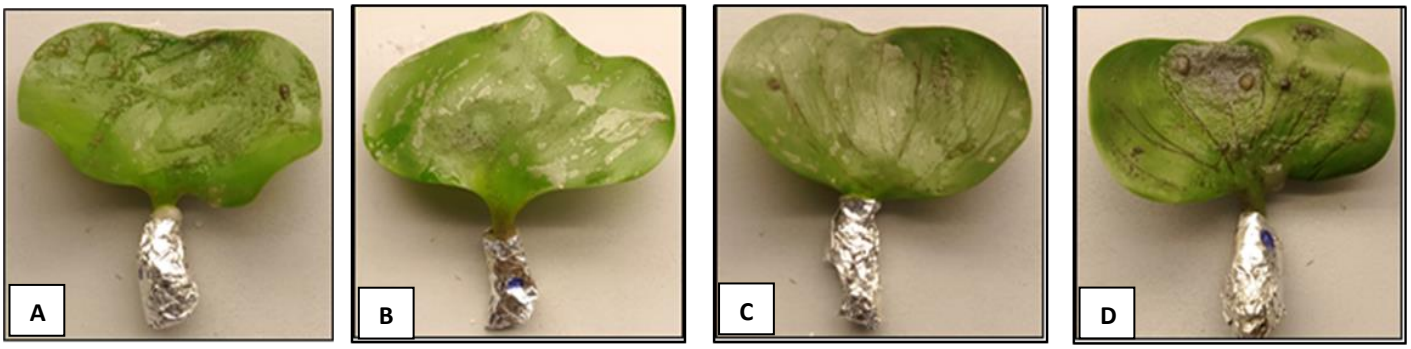
WH-4 [DAY-05]



Fungus(WH-4) + d.H2O | Fungus(WH-4) + T.20 | Scratch Leaf+ Fungus (WH-4) + d.H2O | Scratch Leaf+ Fungus (WH-4) + T.20

Figure7.4.12 | Toxicity assay of fungus (WH-4) observed on day 05 after application of fungus (WH-4) on the healthy leaves of water hyacinth. A| Fungus(WH-4) with distilled water, B| Fungus(WH-4) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-4) and distilled water, D| On Scratch Leaf with Fungus(WH-4) and Tween20 solution.

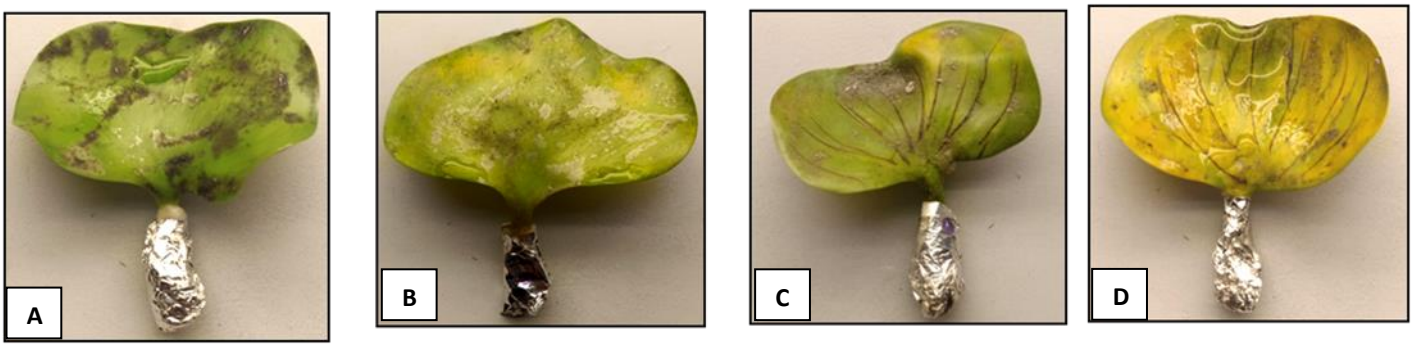
WH-5 [DAY-01]



Fungus(WH-5) + d.H2O | Fungus(WH-5) + T.20 | Scratch Leaf+ Fungus (WH-5) + d.H2O | Scratch Leaf+ Fungus (WH-5) + T.20

Figure7.4.13 | Toxicity assay of fungus (WH-5) observed on day 01 after application of fungus (WH-5) on the healthy leaves of water hyacinth. A| Fungus(WH-5) with distilled water, B| Fungus(WH-5) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-5) and distilled water, D| On Scratch Leaf with Fungus(WH-5) and Tween20 solution.

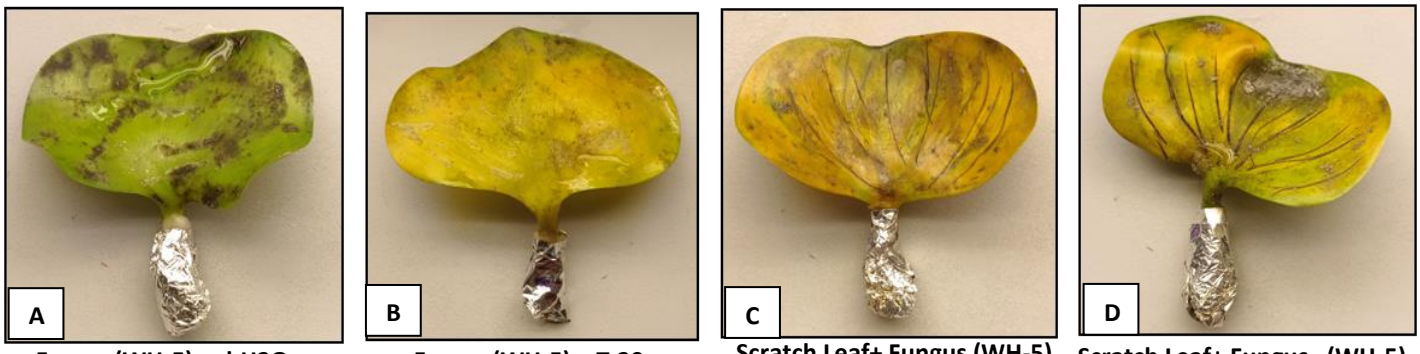
WH-5 [DAY-03]



Fungus(WH-5) + d.H2O | Fungus(WH-5) + T.20 | Scratch Leaf+ Fungus (WH-5) + d.H2O | Scratch Leaf+ Fungus (WH-5) + T.20

Figure7.4.14 | Toxicity assay of fungus (WH-5) observed on day 03 after application of fungus (WH-5) on the healthy leaves of water hyacinth. A| Fungus(WH-5) with distilled water, B| Fungus(WH-5) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-5) and distilled water, D| On Scratch Leaf with Fungus(WH-5) and Tween20 solution.

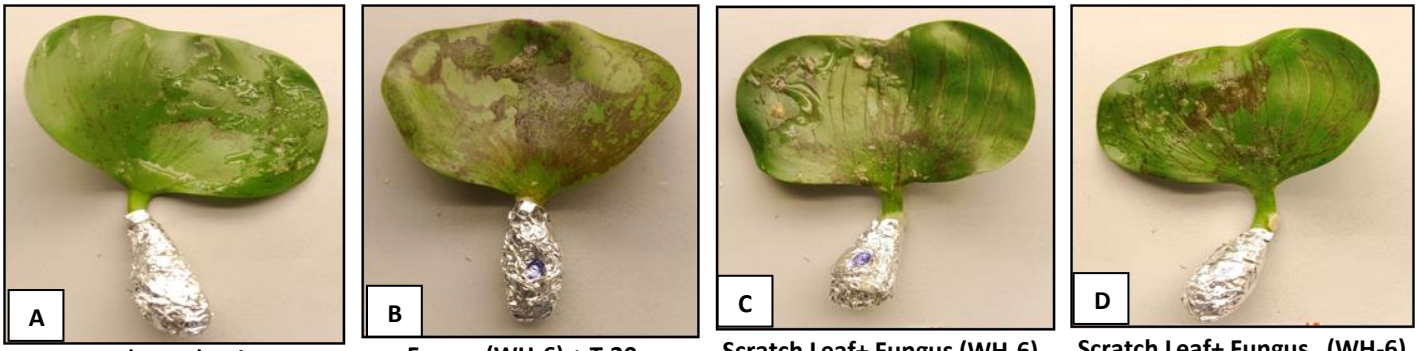
WH-5 [DAY-05]



Fungus(WH-5) + d.H2O | Fungus(WH-5) + T.20 | Scratch Leaf+ Fungus (WH-5) + d.H2O | Scratch Leaf+ Fungus (WH-5) + T.20

Figure7.4.15 | Toxicity assay of fungus (WH-5) observed on day 05 after application of fungus (WH-5) on the healthy leaves of water hyacinth. A| Fungus(WH-5) with distilled water, B| Fungus(WH-5) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-5) and distilled water, D| On Scratch Leaf with Fungus(WH-5) and Tween20 solution.

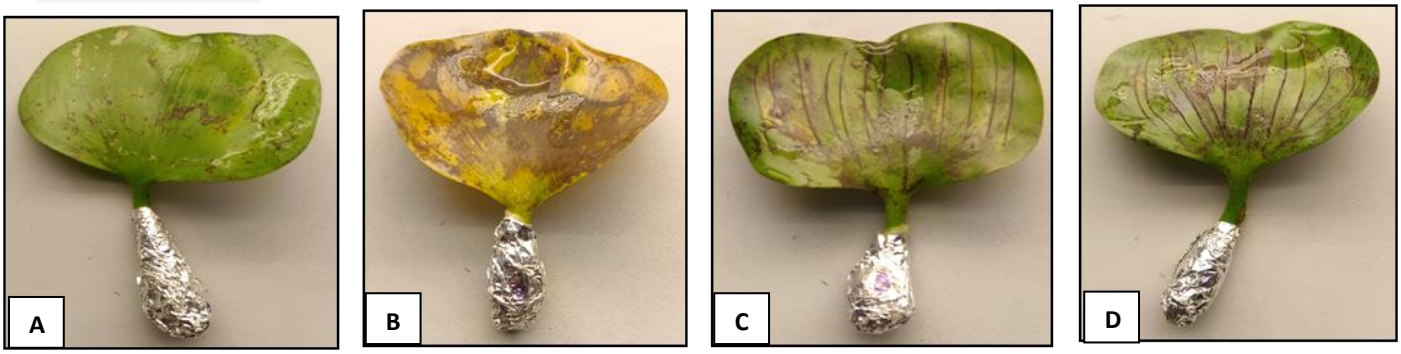
WH-6 [DAY-01]



Fungus(WH-6) + d.H2O | Fungus(WH-6) + T.20 | Scratch Leaf+ Fungus (WH-6) + d.H2O | Scratch Leaf+ Fungus (WH-6) + T.20

Figure7.4.16 | Toxicity assay of fungus (WH-6) observed on day 01 after application of fungus (WH-6) on the healthy leaves of water hyacinth. A| Fungus(WH-6) with distilled water, B| Fungus(WH-6) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-6) and distilled water, D| On Scratch Leaf with Fungus(WH-6) and Tween20 solution.

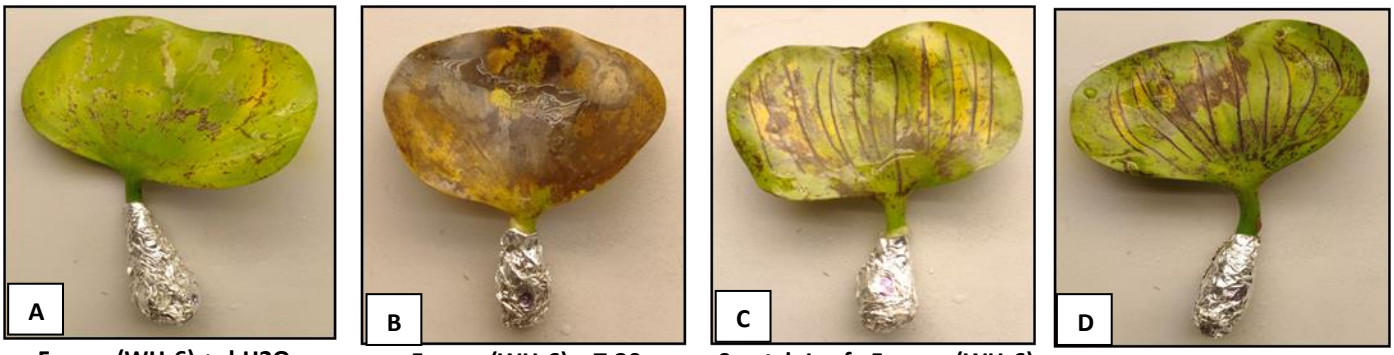
WH-6 [DAY-03]



Fungus(WH-6) + d.H2O | Fungus(WH-6) + T.20 | Scratch Leaf+ Fungus (WH-6) + d.H2O | Scratch Leaf+ Fungus (WH-6) + T.20

Figure7.4.17 | Toxicity assay of fungus (WH-6) observed on day 03 after application of fungus (WH-6) on the healthy leaves of water hyacinth. A| Fungus(WH-6) with distilled water, B| Fungus(WH-6) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-6) and distilled water, D| On Scratch Leaf with Fungus(WH-6) and Tween20 solution.

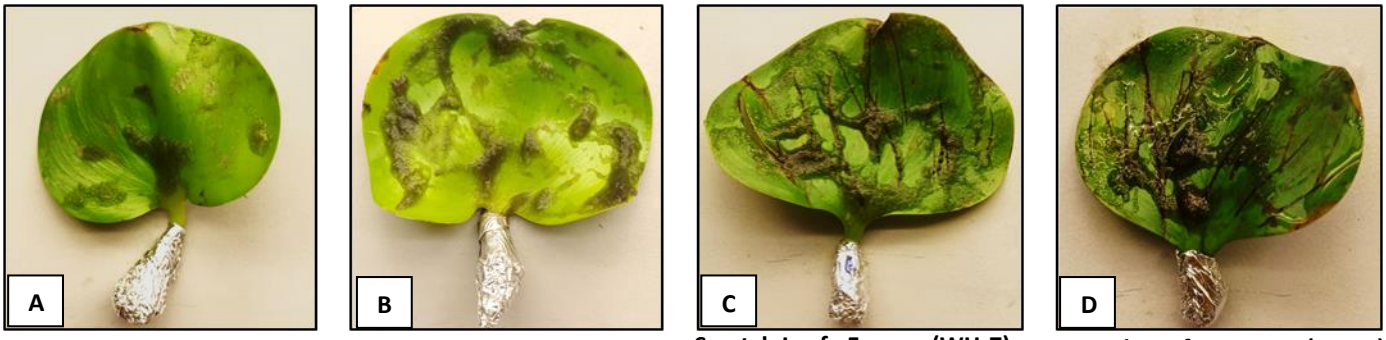
WH-6 [DAY-05]



Fungus(WH-6) + d.H2O | Fungus(WH-6) + T.20 | Scratch Leaf+ Fungus (WH-6) + d.H2O | Scratch Leaf+ Fungus (WH-6) + T.20

Figure7.4.18 | Toxicity assay of fungus (WH-6) observed on day 05 after application of fungus (WH-6) on the healthy leaves of water hyacinth. A| Fungus(WH-6) with distilled water, B| Fungus(WH-6) with Tween20 solution C| On Scratch Leaf with Fungus(WH-6) and distilled water, D| On Scratch Leaf with Fungus(WH-6) and Tween20 solution.

WH-7 [DAY-01]



Fungus(WH-7) + d.H2O | Fungus(WH-7) + T.20 | Scratch Leaf+ Fungus (WH-7) + d.H2O | Scratch Leaf+ Fungus (WH-7) + T.20

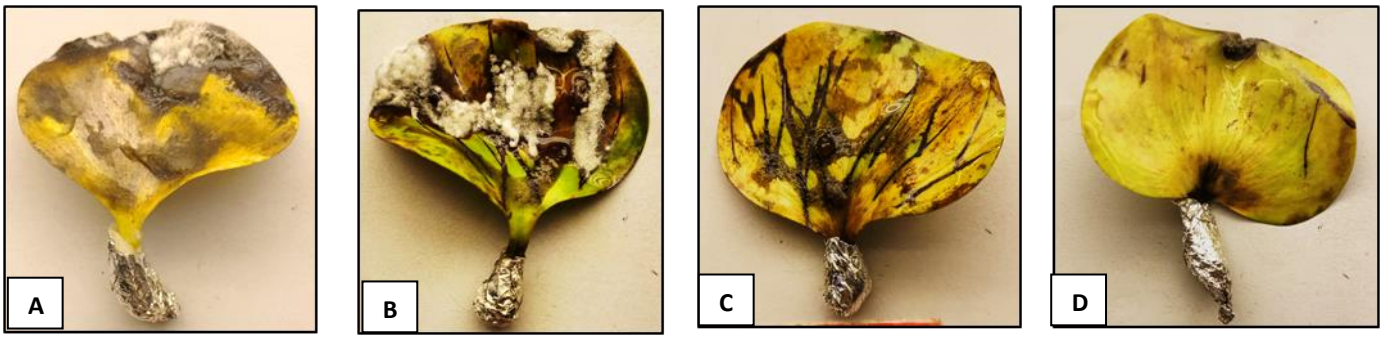
Figure7.4.19 | Toxicity assay of fungus (WH-7) observed on day 01 after application of fungus (WH-7) on the healthy leaves of water hyacinth. A| Fungus(WH-7) with distilled water, B| Fungus(WH-7) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-7) and distilled water, D| On Scratch Leaf with Fungus(WH-7) and Tween20 solution.

WH-7 [DAY-03]

Fungus(WH-7) + d.H2O | Fungus(WH-7) + T.20 | Scratch Leaf+ Fungus (WH-7) + d.H2O | Scratch Leaf+ Fungus (WH-7) + T.20

Figure7.4.20 | Toxicity assay of fungus (WH-7) observed on day 03 after application of fungus (WH-7) on the healthy leaves of water hyacinth. A| Fungus(WH-7) with distilled water, B| Fungus(WH-7) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-7) and distilled water, D| On Scratch Leaf with Fungus(WH-7) and Tween20 solution.

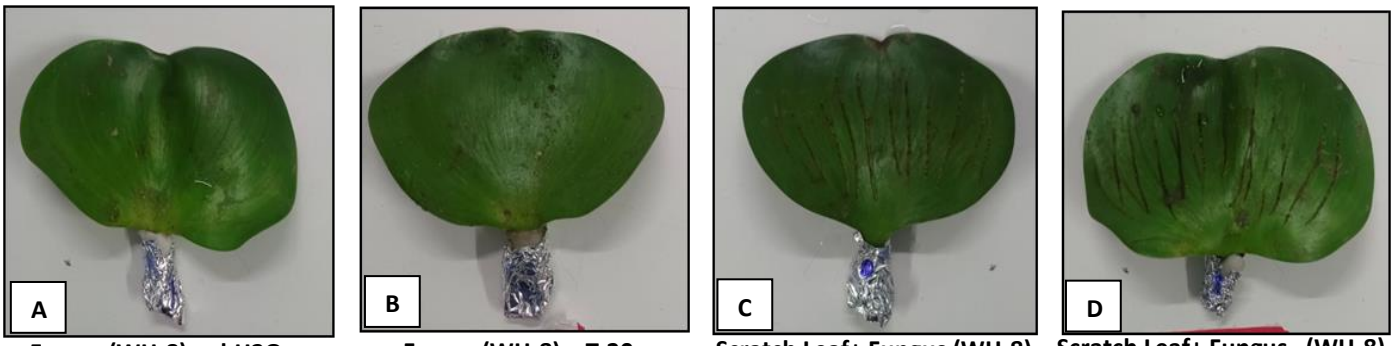
WH-7 [DAY-05]



Fungus(WH-7) + d.H2O | Fungus(WH-7) + T.20 | Scratch Leaf+ Fungus (WH-7) + d.H2O | Scratch Leaf+ Fungus (WH-7) + T.20

Figure7.4.21 | Toxicity assay of fungus (WH-7) observed on day 05 after application of fungus (WH-7) on the healthy leaves of water hyacinth. A| Fungus(WH-7) with distilled water, B| Fungus(WH-7) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-7) and distilled water, D| On Scratch Leaf with Fungus(WH-7) and Tween20 solution.

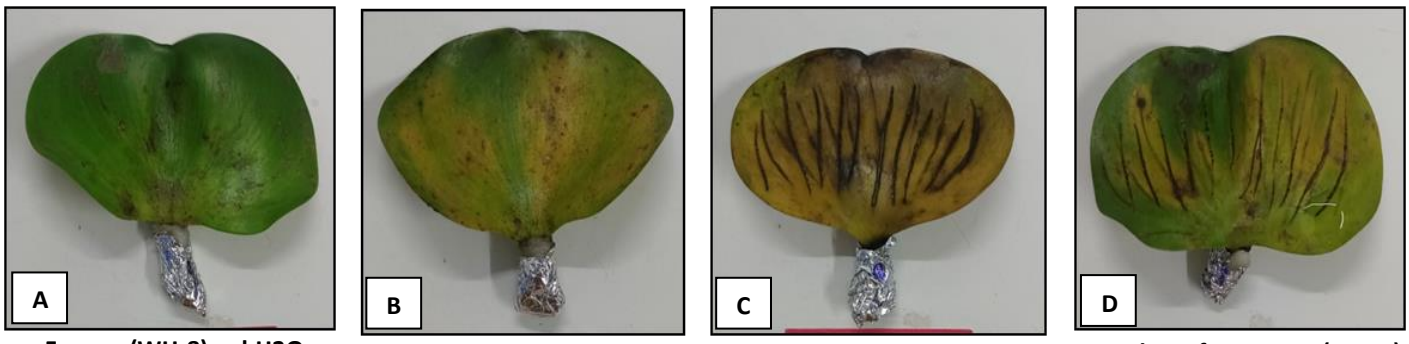
WH-8 [DAY-01]



Fungus(WH-8) + d.H2O | Fungus(WH-8) + T.20 | Scratch Leaf+ Fungus (WH-8) + d.H2O | Scratch Leaf+ Fungus (WH-8) + T.20

Figure7.4.22 | Toxicity assay of fungus (WH-8) observed on day 01 after application of fungus (WH-8) on the healthy leaves of water hyacinth. A| Fungus(WH-8) with distilled water, B| Fungus(WH-8) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-8) and distilled water, D| On Scratch Leaf with Fungus(WH-8) and Tween20 solution.

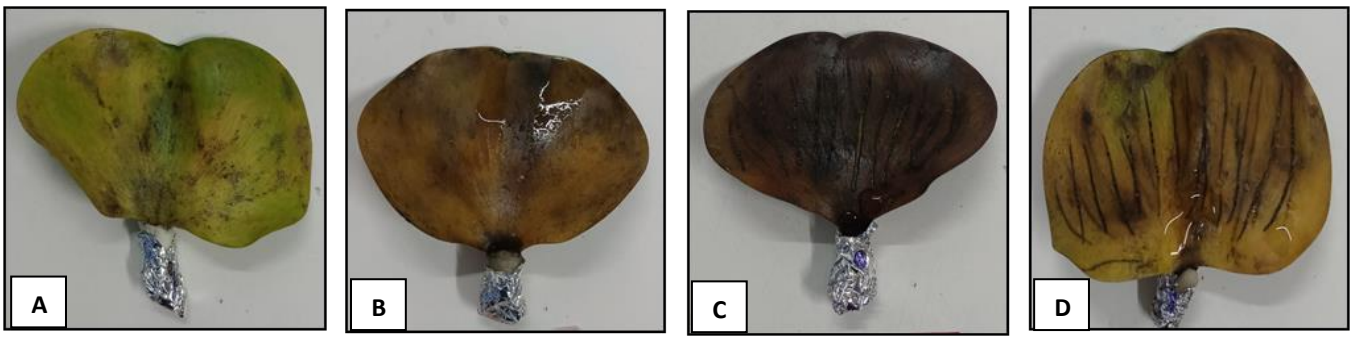
WH-8 [DAY-03]



Fungus (WH-8) + d.H2O | Fungus (WH-8) + T.20 | Scratch Leaf+ Fungus (WH-1) + d.H2O | Scratch Leaf+ Fungus (WH-1) + T.20

Figure7.4.23 | Toxicity assay of fungus (WH-8) observed on day 03 after application of fungus (WH-8) on the healthy leaves of water hyacinth. A| Fungus(WH8) with distilled water, B| Fungus(WH-8) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-8) and distilled water, D| On Scratch Leaf with Fungus(WH-8) and Tween20 solution.

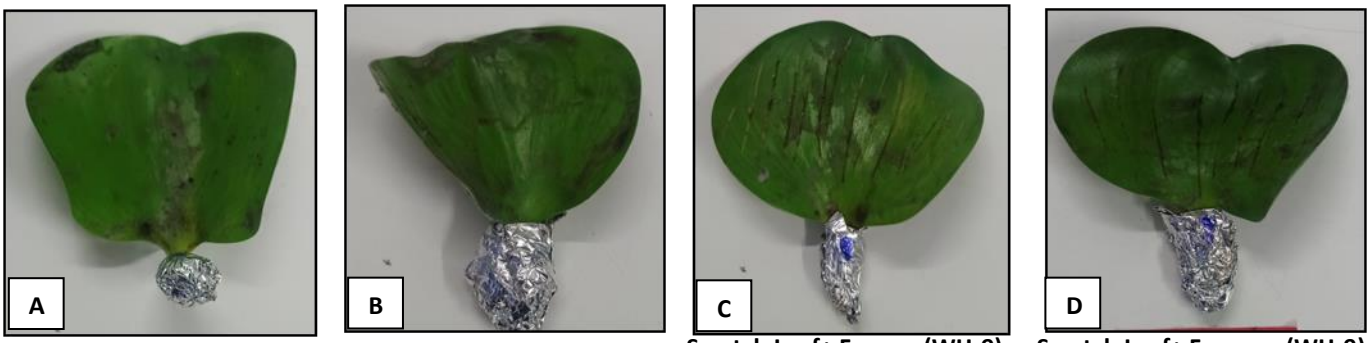
WH-8 [DAY-05]



Fungus (WH-8) + d.H2O | Fungus (WH-8) + T.20 | Scratch Leaf+ Fungus (WH-8) + d.H2O | Scratch Leaf+ Fungus (WH-8) + T.20

Figure7.4.24 | Toxicity assay of fungus (WH-8) observed on day 05 after application of fungus (WH-8) on the healthy leaves of water hyacinth. A| Fungus(WH-8) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-8) and distilled water, D| On Scratch Leaf with Fungus(WH-8) and Tween20 solution.

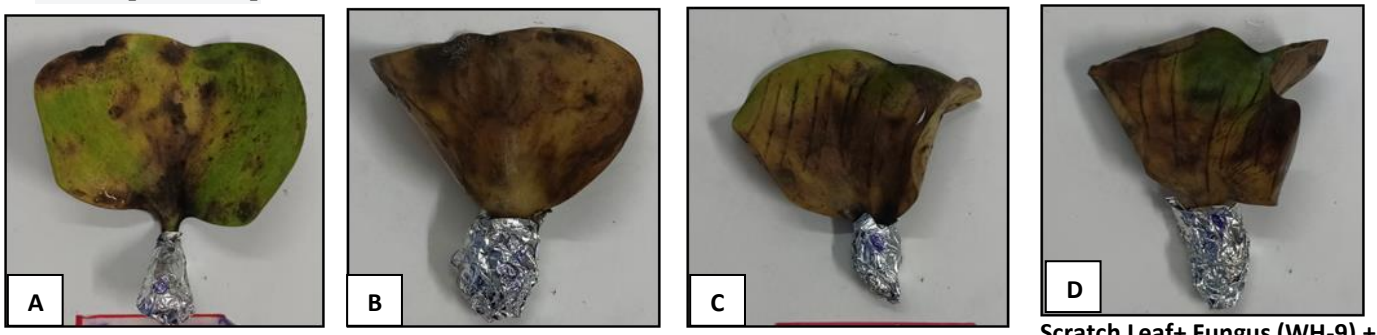
W H-9 [DAY-01]



Fungus(WH-9) + d.H2O | Fungus (WH-9) + T.20 | Scratch Leaf+ Fungus (WH-9) + d.H2O | Scratch Leaf+ Fungus (WH-9) + T.20

Figure7.4.25 | Toxicity assay of fungus (WH-9) observed on day 01 after application of fungus (WH-9) on the healthy leaves of water hyacinth. A| Fungus(WH-9) with distilled water, B| Fungus(WH-9) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-9) and distilled water, D| On Scratch Leaf with Fungus(WH-9) and Tween20 solution.

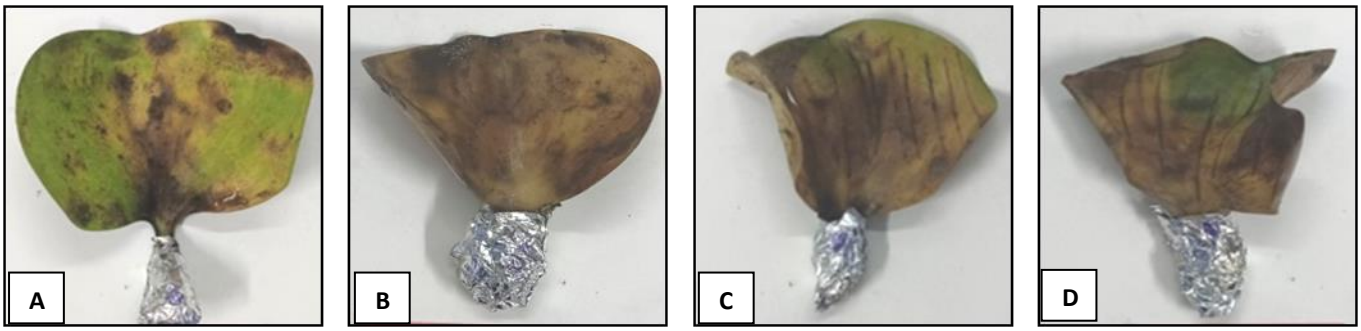
WH-9 [DAY-03]



Fungus(WH-9) + d.H2O | Fungus (WH-9) + T.20 | Scratch Leaf+ Fungus (WH-9) + d.H2O | Scratch Leaf+ Fungus (WH-9) + T.20

Figure7.4.26 | Toxicity assay of fungus (WH-9) observed on day 03 after application of fungus (WH-9) on the healthy leaves of water hyacinth. A| Fungus(WH-9) with distilled water, B| Fungus(WH-9) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-9) and distilled water, D| On Scratch Leaf with Fungus(WH-9) and Tween20 solution.

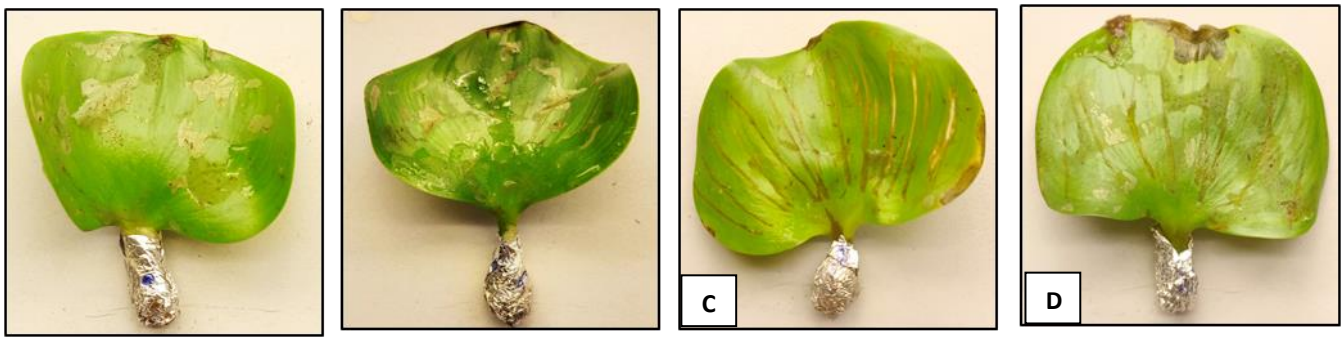
W H-9 [DAY-05]



Fungus(WH-9) + d.H2O | Fungus (WH-9) + T.20 | Scratch Leaf+ Fungus (WH-9) + d.H2O | Scratch Leaf+ Fungus (WH-9) + T.20

Figure7.4.27 | Toxicity assay of fungus (WH-9) observed on day 05 after application of fungus (WH-9) on the healthy leaves of water hyacinth. A| Fungus(WH-9) with distilled water, B| Fungus(WH-9) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-9) and distilled water, D| On Scratch Leaf with Fungus(WH-9) and Tween20 solution.

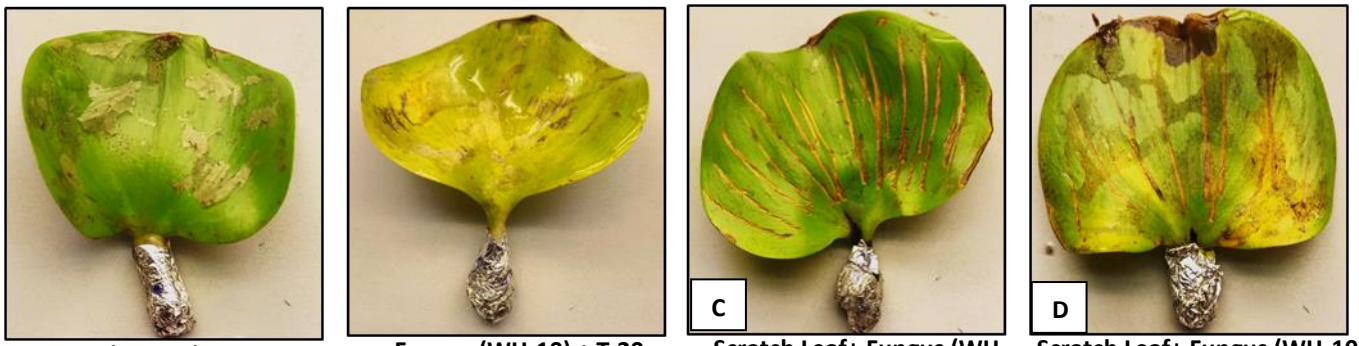
W H-10 [DAY-01]



Fungus (WH-10)+d.H2O | Fungus (WH-10) + T.20 | Scratch Leaf+ Fungus (WH-10) + d.H2O | Scratch Leaf+ Fungus (WH-10) + T.20

Figure7.4.28 | Toxicity assay of fungus (WH-10) observed on day 01 after application of fungus (WH-10) on the healthy leaves of water hyacinth. A| Fungus(WH-10) with distilled water, B| Fungus (WH-10) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-10) and distilled water, D| On Scratch Leaf with Fungus(WH-10) and Tween20 solution.

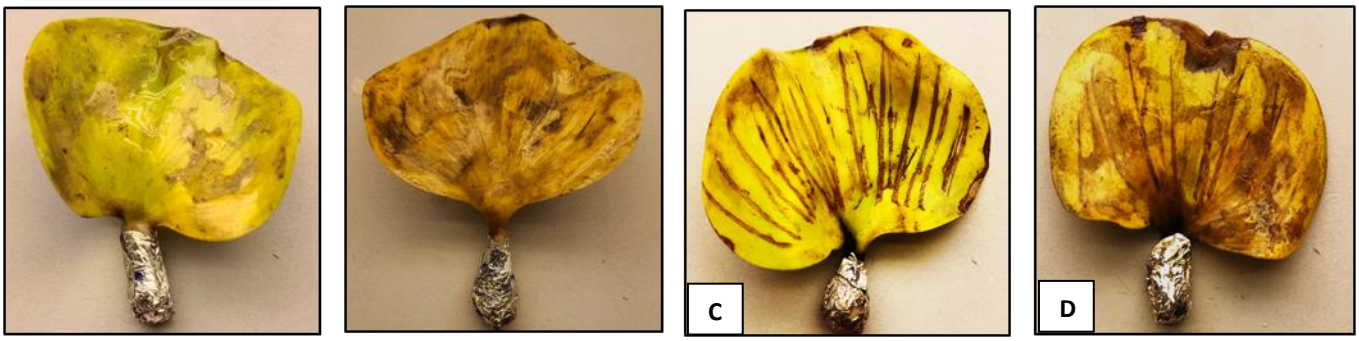
W H-10 [DAY-03]



Fungus(WH-10) + d.H2O | Fungus (WH-10) + T.20 | Scratch Leaf+ Fungus (WH-10) + d.H2O | Scratch Leaf+ Fungus (WH-10) + T.20

Figure7.4.29 | Toxicity assay of fungus (WH-10) observed on day 03 after application of fungus (WH-10) on the healthy leaves of water hyacinth. A| Fungus(WH-10) with distilled water, B| Fungus(WH-10) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-10) and distilled water, D| On Scratch Leaf with Fungus(WH-10) and Tween20 solution.

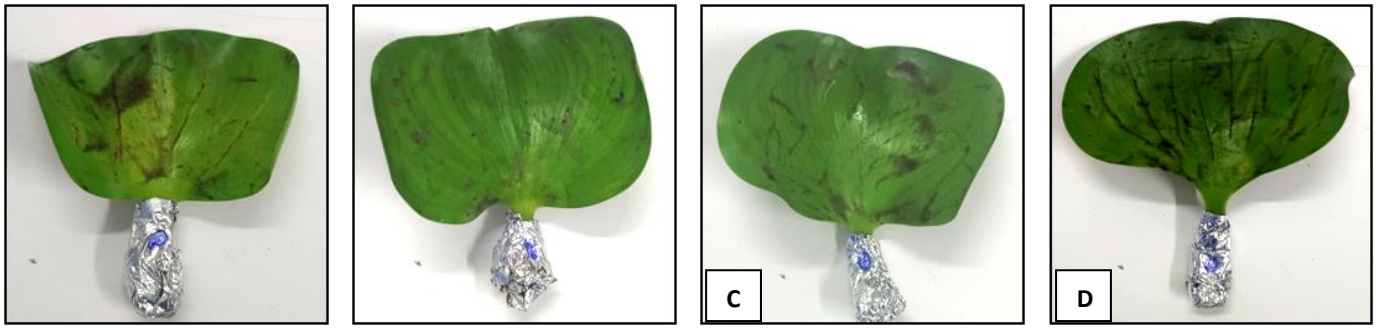
W H-10 [DAY-05]



Fungus(WH-10) + d.H2O | Fungus (WH-10) + T.20 | Scratch Leaf+ Fungus (WH-10) + d.H2O | Scratch Leaf+ Fungus (WH-10) + T.20

Figure7.4.30 | Toxicity assay of fungus (WH-10) observed on day 05 after application of fungus (WH-10) on the healthy leaves of water hyacinth. A| Fungus(WH-10) with distilled water, B| Fungus(WH-10) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-10) and distilled water, D| On Scratch Leaf with Fungus(WH-10) and Tween20 solution.

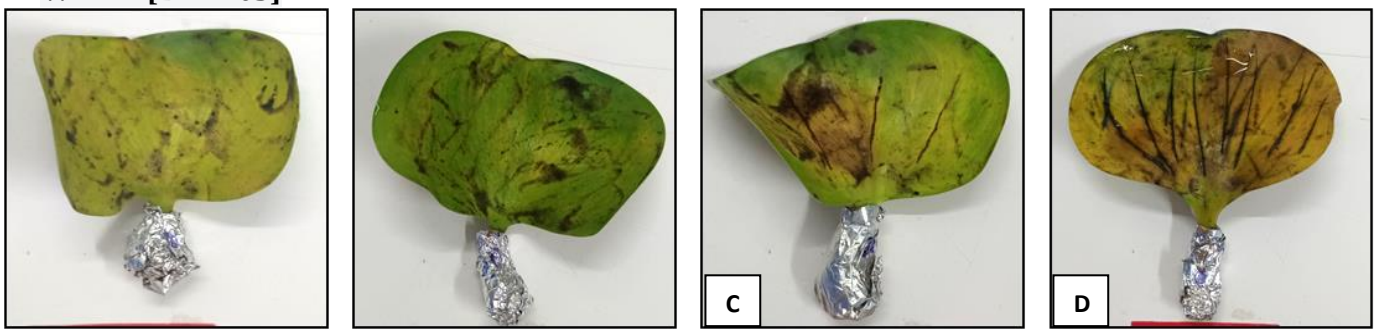
WH-12 [DAY-01]



Fungus(WH-12) + d.H2O | Fungus (WH-12) + T.20 | Scratch Leaf+ Fungus (WH-12) + d.H2O | Scratch Leaf+ Fungus (WH-12) + T.20

Figure7.4.31| Toxicity assay of fungus (WH-12) observed on day 01 after application of fungus (WH-12) on the healthy leaves of water hyacinth. A| Fungus(WH-12) with distilled water, B| Fungus(WH-12) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-12) and distilled water, D| On Scratch Leaf with Fungus(WH-12) and Tween20 solution.

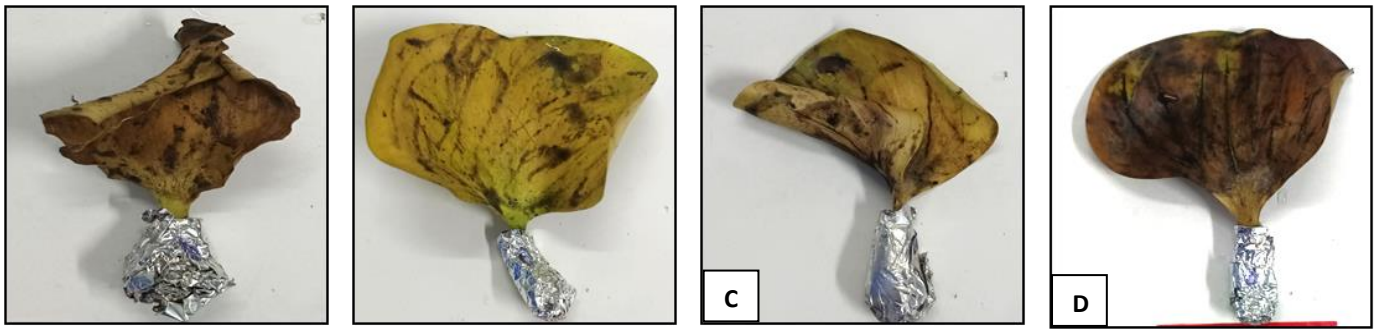
WH-12 [DAY-03]



Fungus(WH-12) + d.H2O | Fungus (WH-12) + T.20 | Scratch Leaf+ Fungus (WH-12) + d.H2O | Scratch Leaf+ Fungus (WH-12) + T.20

Figure7.4.32 | Toxicity assay of fungus (WH-12) observed on day 03 after application of fungus (WH-12) on the healthy leaves of water hyacinth. A| Fungus(WH-12) with distilled water, B| Fungus(WH-12) with Tween20 solution C| On Scratch Leaf with Fungus(WH-12) and distilled water, D| On Scratch Leaf with Fungus(WH-1) and Tween20 solution.

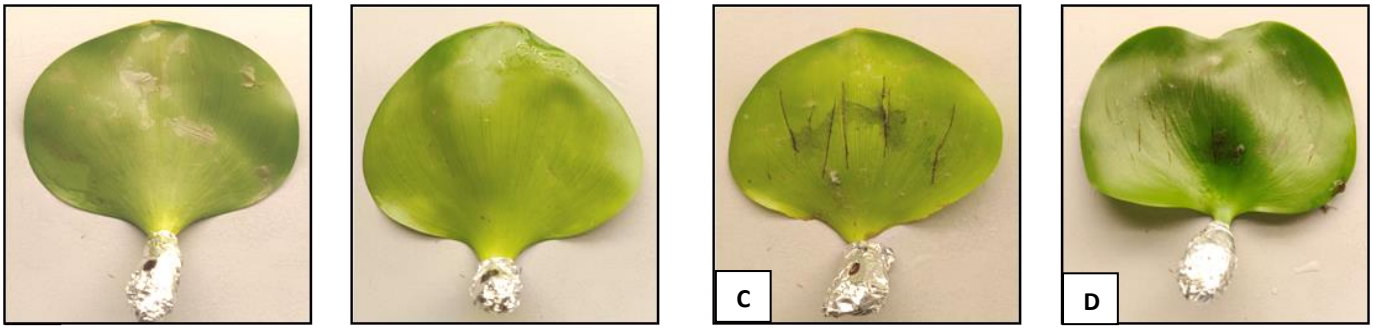
WH-12 [DAY-05]



Fungus(WH-12) + d.H2O | Fungus (WH-12) + T.20 | Scratch Leaf+ Fungus (WH-12) + d.H2O | Scratch Leaf+ Fungus (WH-12) + T.20

Figure7.4.33 | Toxicity assay of fungus (WH-12) observed on day 05 after application of fungus (WH-12) on the healthy leaves of water hyacinth. A| Fungus(WH-12) with distilled water, B| Fungus(WH-12) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-12) and distilled water, D| On Scratch Leaf with Fungus(WH-12) and Tween20 solution.

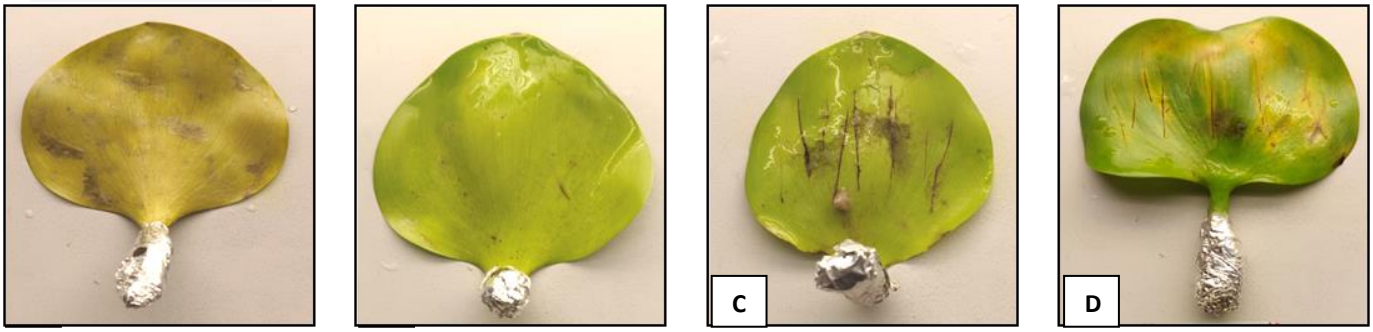
WH-13 [DAY-01]



Fungus(WH-13) + d.H2O | Fungus (WH-13) + T.20 | Scratch Leaf+ Fungus (WH-13) + d.H2O | Scratch Leaf+ Fungus (WH-13) + T.20

Figure7.4.34 | Toxicity assay of fungus (WH-13) observed on day 01 after application of fungus (WH-13) on the healthy leaves of water hyacinth. A| Fungus(WH-13) with distilled water, B| Fungus(WH-13) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-13) and distilled water, D| On Scratch Leaf with Fungus(WH-13) and Tween20 solution.

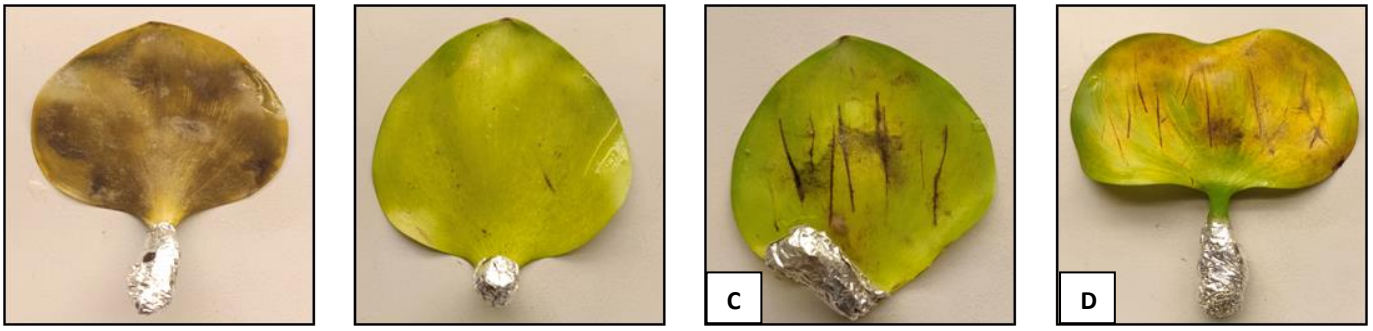
WH-13 [DAY-03]



Fungus(WH-13) + d.H2O | Fungus (WH-13) + T.20 | Scratch Leaf+ Fungus (WH-13) + d.H2O | Scratch Leaf+ Fungus (WH-13) + T.20

Figure7.4.35 | Toxicity assay of fungus (WH-13) observed on day 03 after application of fungus (WH-13) on the healthy leaves of water hyacinth. A| Fungus(WH-13) with distilled water, B| Fungus(WH-13) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-13) and distilled water, D| On Scratch Leaf with Fungus(WH-13) and Tween20 solution.

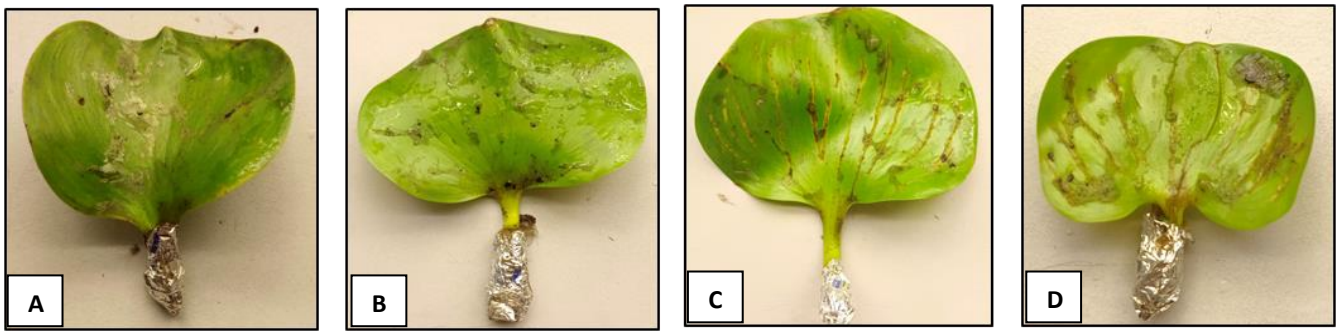
WH-13 [DAY-05]



Fungus(WH-13) + d.H2O | Fungus (WH-13) + T.20 | Scratch Leaf+ Fungus (WH-13) + d.H2O | Scratch Leaf+ Fungus (WH-13) + T.20

Figure7.4.36 | Toxicity assay of fungus (WH-13) observed on day 05 after application of fungus (WH-13) on the healthy leaves of water hyacinth. A| Fungus(WH-13) with distilled water, B| Fungus(WH-13) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-13) and distilled water, D| On Scratch Leaf with Fungus(WH-13) and Tween20 solution.

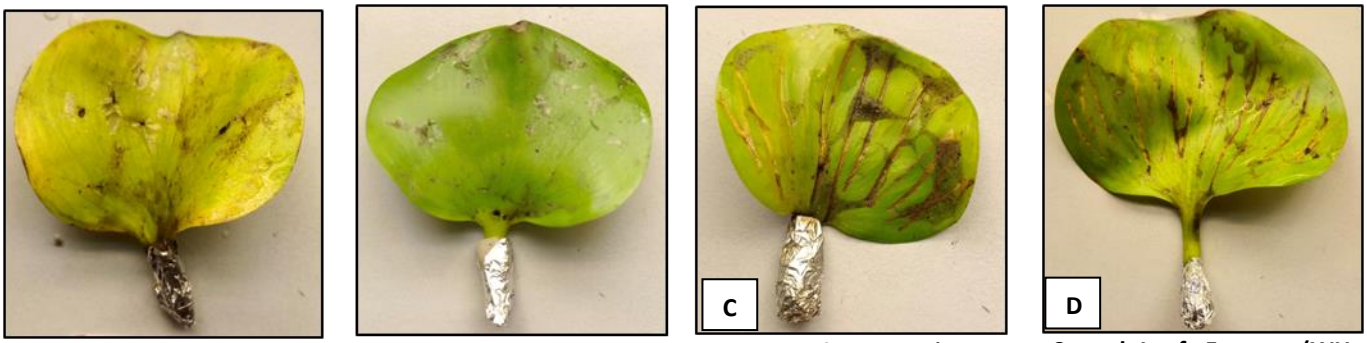
WH-14 [DAY-01]



Fungus(WH-14) + d.H2O | Fungus (WH-14) + | Scratch Leaf+ Fungus (WH-14) + d.H2O | Scratch Leaf+ Fungus (WH-14) + T.20

Figure7.4.37| Toxicity assay of fungus (WH-14) observed on day 01 after application of fungus (WH-14) on the healthy leaves of water hyacinth. A| Fungus(WH-14) with distilled water, B| Fungus(WH-14) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-14) and distilled water, D| On Scratch Leaf with Fungus(WH-14) and Tween20 solution.

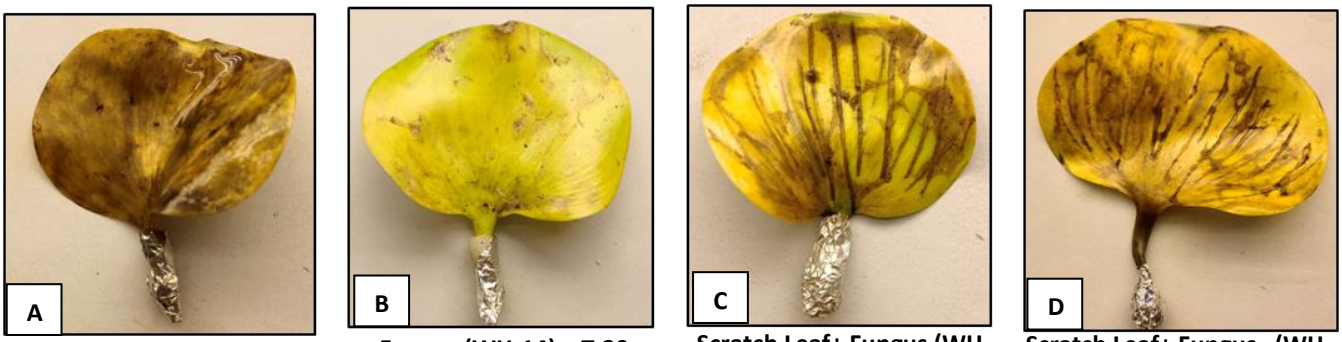
WH-14 [DAY-03]



Fungus(WH-14) + d.H2O | Fungus (WH-14) + T.20 | Scratch Leaf+ Fungus (WH-14) + d.H2O | Scratch Leaf+ Fungus (WH-14) + T.20

Figure7.4.38 | Toxicity assay of fungus (WH-14) observed on day 03 after application of fungus (WH-14) on the healthy leaves of water hyacinth. A| Fungus(WH-14) with distilled water, B| Fungus(WH-14) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-14) and distilled water, D| On Scratch Leaf with Fungus(WH-14) and Tween20 solution.

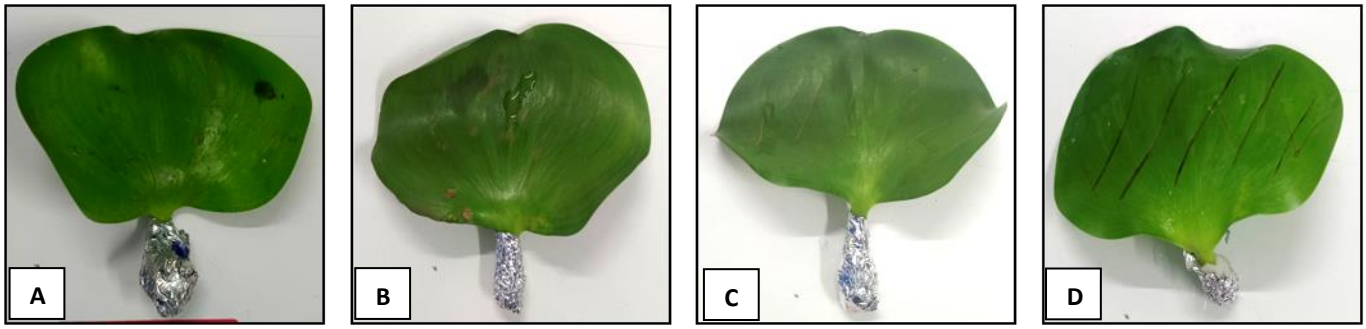
WH-14 [DAY-05]



Fungus(WH-14) + d.H2O | Fungus (WH-14) + T.20 | Scratch Leaf+ Fungus (WH-14) + d.H2O | Scratch Leaf+ Fungus (WH-14) + T.20

Figure7.4.39 | Toxicity assay of fungus (WH-14) observed on day 05 after application of fungus (WH-14) on the healthy leaves of water hyacinth. A| Fungus(WH-14) with distilled water, B| Fungus(WH-14) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-14) and distilled water, D| On Scratch Leaf with Fungus(WH-14) and Tween20 solution.

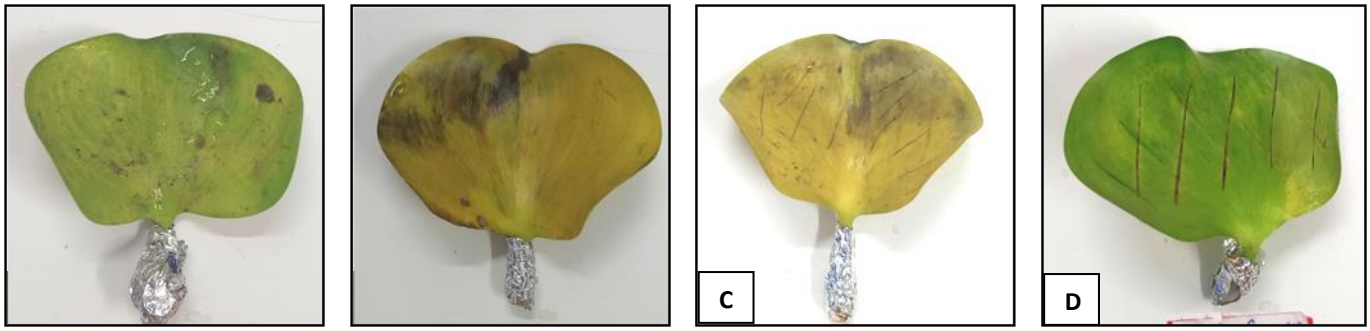
WH-15 [DAY-01]



Fungus(WH-15) + d.H2O | Fungus (WH-15) + T.20 | Scratch Leaf+ Fungus (WH-15) + d.H2O | Scratch Leaf+ Fungus (WH-15) + T.20

Figure7.4.40 | Toxicity assay of fungus (WH-15) observed on day 01 after application of fungus (WH-15) on the healthy leaves of water hyacinth. A| Fungus(WH1-5) with distilled water, B| Fungus(WH-15) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-15) and distilled water, D| On Scratch Leaf with Fungus(WH-15) and Tween20 solution.

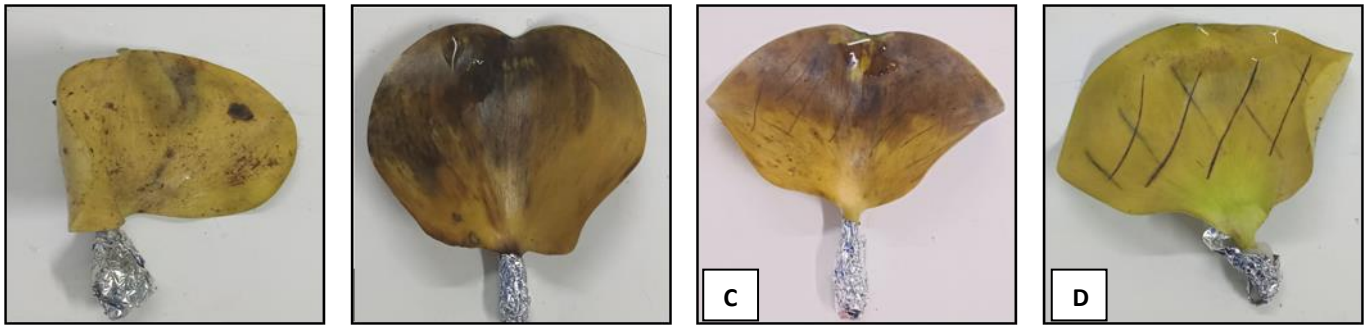
WH-15 [DAY-03]



Fungus (WH-15) + d.H2O | Fungus (WH-15) + T.20 | Scratch Leaf+ Fungus (WH-15) + d.H2O | Scratch Leaf+ Fungus (WH-15) + T.20

Figure7.4.41 | Toxicity assay of fungus (WH-15) observed on day 03 after application of fungus (WH-15) on the healthy leaves of water hyacinth. A| Fungus(WH-15) with distilled water, B| Fungus(WH-15) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-15) and distilled water, D| On Scratch Leaf with Fungus(WH-15) and Tween20 solution.

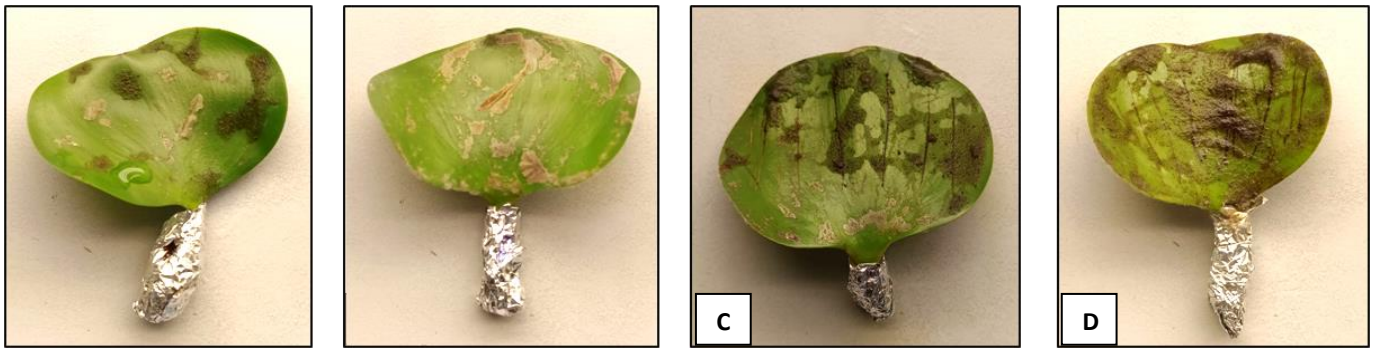
WH-15 [DAY-05]



Fungus (WH-15) + d.H2O | Fungus (WH-15) + T.20 | Scratch Leaf+ Fungus (WH-15) + d.H2O | Scratch Leaf+ Fungus (WH-15) + T.20

Figure7.4.42 | Toxicity assay of fungus (WH-15) observed on day 05 after application of fungus (WH-15) on the healthy leaves of water hyacinth. A| Fungus(WH-15) with distilled water, B| Fungus(WH-15) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-15) and distilled water, D| On Scratch Leaf with Fungus(WH-15) and Tween20 solution.

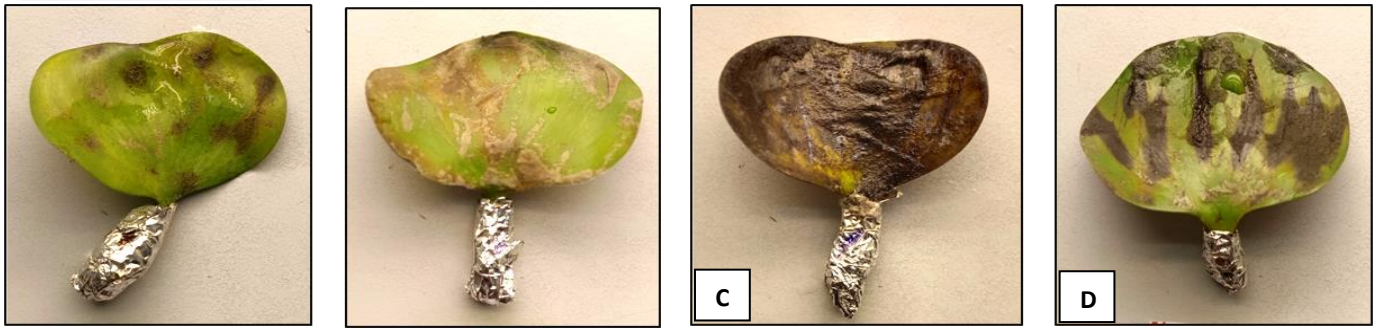
WH-18 [DAY-01]



Fungus (WH-18) + d.H2O | Fungus (WH-18) + T.20 | Scratch Leaf+ Fungus (WH-18) + d.H2O | Scratch Leaf+ Fungus (WH-18) + T.20

Figure7.4.43 | Toxicity assay of fungus (WH-18) observed on day 01 after application of fungus (WH-18) on the healthy leaves of water hyacinth. A| Fungus (WH-18) with distilled water, B| Fungus(WH-18) with Tween20 solution ,C| On Scratch Leaf with Fungus (WH-18) and distilled water, D| On Scratch Leaf with Fungus(WH-18) and Tween20 solution.

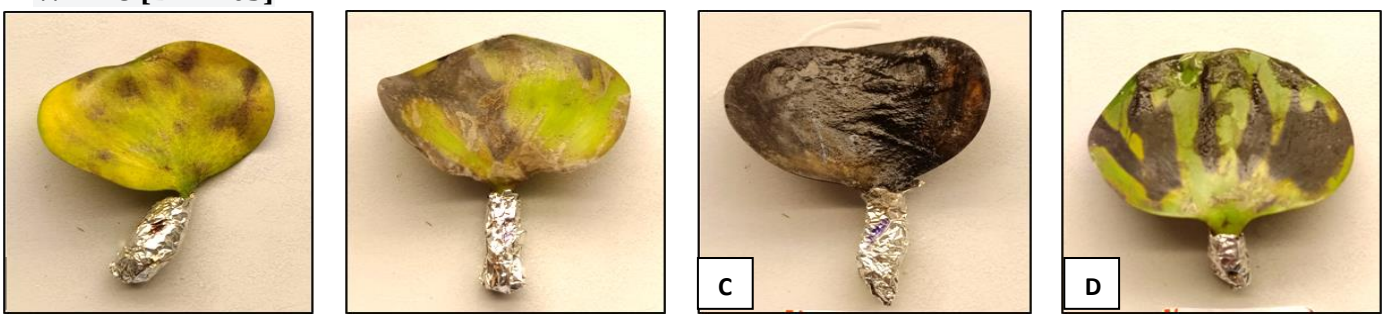
WH-18 [DAY-03]



Fungus(WH-18) + d.H2O | Fungus (WH-18) + T.20 | Scratch Leaf+ Fungus (WH-18) + d.H2O | Scratch Leaf+ Fungus (WH-18) + T.20

Figure7.4.44 | Toxicity assay of fungus (WH-18) observed on day 03 after application of fungus (WH-18) on the healthy leaves of water hyacinth. A| Fungus(WH-18) with distilled water, B| Fungus(WH-18) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-18) and distilled water, D| On Scratch Leaf with Fungus(WH-18) and Tween20 solution.

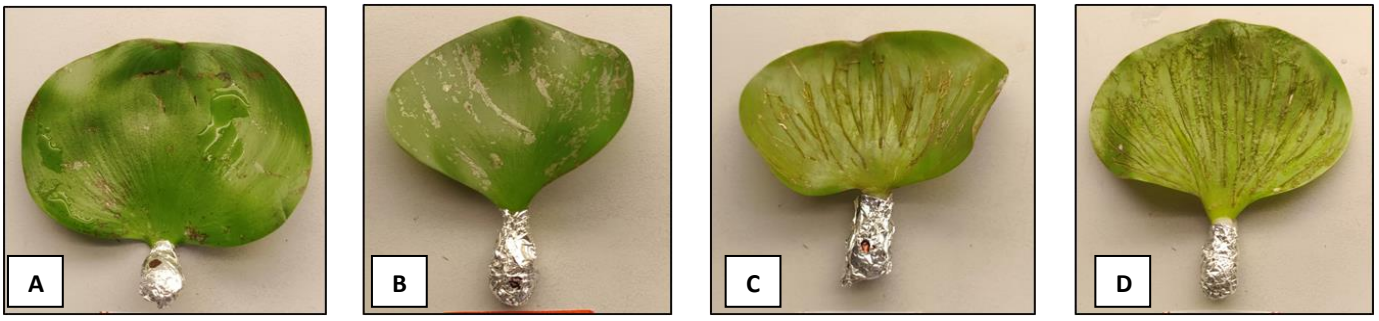
WH-18 [DAY-05]



Fungus (WH-18) + d.H2O | Fungus (WH-18) + T.20 | Scratch Leaf+ Fungus (WH-18) + d.H2O | Scratch Leaf+ Fungus (WH-18) + T.20

Figure7.4.45 | Toxicity assay of fungus (WH-18) observed on day 05 after application of fungus (WH-18) on the healthy leaves of water hyacinth. A| Fungus(WH-18) with distilled water, B| Fungus(WH-18) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-18) and distilled water, D| On Scratch Leaf with Fungus(WH-18) and Tween20 solution.

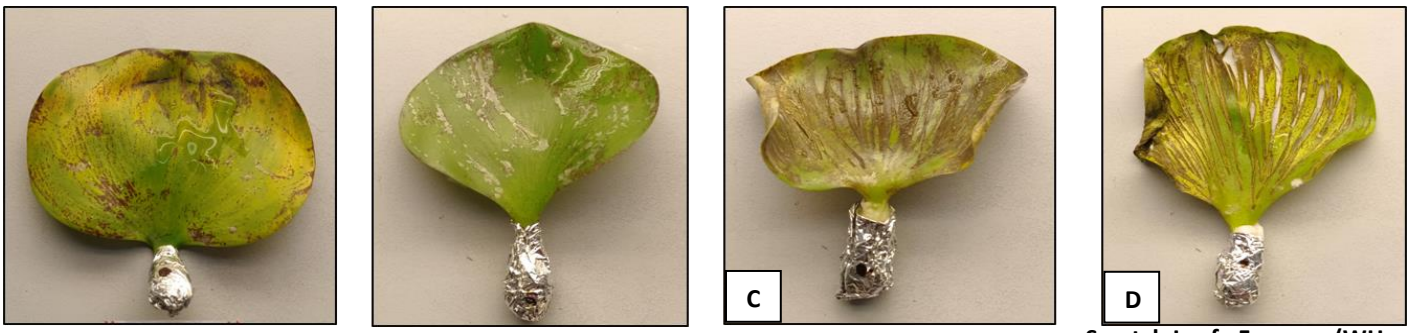
WH-19 [DAY-01]



Fungus (WH-19) + d.H2O | Fungus (WH-19) + T.20 | Scratch Leaf+ Fungus (WH-19) + d.H2O | Scratch Leaf+ Fungus (WH-19) + T.20

Figure7.4.46 | Toxicity assay of fungus (WH-19) observed on day 01 after application of fungus (WH-19) on the healthy leaves of water hyacinth. A| Fungus(WH-19) with distilled water, B| Fungus(WH-19) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-19) and distilled water, D| On Scratch Leaf with Fungus(WH-19) and Tween20 solution.

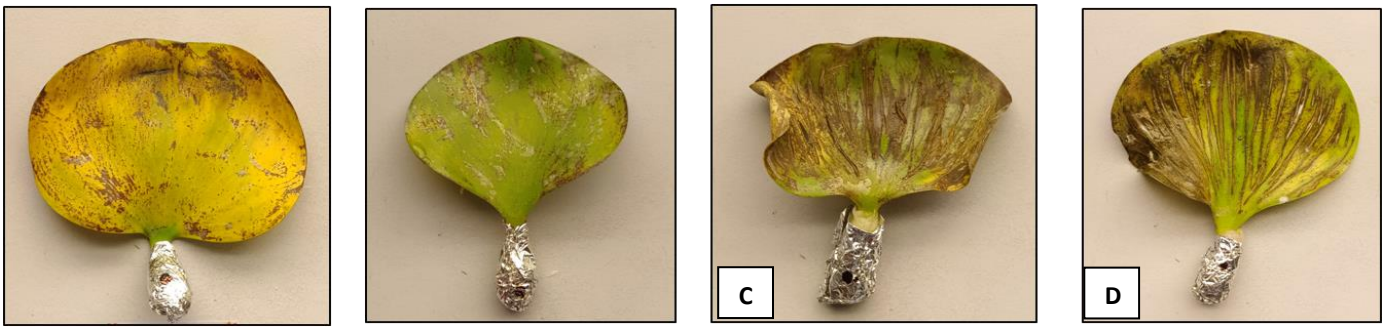
WH-19 [DAY-03]



Fungus (WH-19) + d.H2O | Fungus (WH-19) + T.20 | Scratch Leaf+ Fungus (WH-19) + d.H2O | Scratch Leaf+ Fungus (WH-19) + T.20

Figure7.4.47 | Toxicity assay of fungus (WH-19) observed on day 03 after application of fungus (WH-19) on the healthy leaves of water hyacinth. A| Fungus(WH-19) with distilled water, B| Fungus(WH-19) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-19) and distilled water, D| On Scratch Leaf with Fungus(WH-19) and Tween20 solution.

WH-19 [DAY-05]



Fungus(WH-19) + d.H2O | Fungus (WH-19) + T.20 | Scratch Leaf+ Fungus (WH-19) + d.H2O | Scratch Leaf+ Fungus (WH-19) + T.20

Figure7.4.48 | Toxicity assay of fungus (WH-19) observed on day 05 after application of fungus (WH-19) on the healthy leaves of water hyacinth. A| Fungus(WH-19) with distilled water, B| Fungus(WH-1) with Tween20 solution ,C| On Scratch Leaf with Fungus(WH-19) and distilled water, D| On Scratch Leaf with Fungus(WH-19) and Tween20 solution.

A]

FUNGAL STRAIN NUMBERS	DAY-01			
	Fungus+ D.H20	Fungus+ T 20	S.leaf + Fungus + D.H2O	S.leaf + Fungus + T 20
WH-1	0	0	0	0
WH-2	0	0	0	0
WH-3	1	1	0	1
WH-4	0	0	0	1
WH-5	0	1	0	1
WH-6	0	0	0	0
WH-7	0	0	0	0
WH-8	0	0	0	0
WH-9	0	0	0	0
WH-10	0	0	1	1
WH-12	0	0	0	0
WH-13	0	0	1	1
WH-14	0	0	0	0
WH-15	0	0	0	0
WH-18	0	0	0	0
WH-19	0	0	0	0
CONTROL	0	0	0	0

D. H_2O – distilled water

S. Leaf – Scratch leaf

B]

FUNGAL STRAIN NUMBERS	DAY-03			
	Fungus+ D.H20	Fungus+ T 20	S.leaf + Fungus + D.H2O	S.leaf + Fungus + T 20
WH-1	1	1	1	1
WH-2	1	1	1	0
WH-3	1	1	1	2
WH-4	0	1	1	1
WH-5	0	1	1	0
WH-6	0	1	0	0
WH-7	1	2	1	1
WH-8	0	1	1	1
WH-9	1	2	2	2
WH-10	0	1	1	2
WH-12	0	0	1	1
WH-13	0	0	1	1
WH-14	1	0	1	1
WH-15	0	1	1	0
WH-18	0	1	2	1
WH-19	1	0	1	1
CONTROL	0	0	0	0

C]

FUNGAL STRAIN NUMBERS	DAY-05			
	Fungus+ D.H20	Fungus+ T 20	S.leaf + Fungus + D.H2O	S.leaf + Fungus + T 20
WH-1	2	2	2	2
WH-2	2	2	2	1
WH-3	2	2	2	2
WH-4	2	1	1	1
WH-5	1	0	1	1
WH-6	1	2	2	2
WH-7	2	2	2	2
WH-8	1	2	2	1
WH-9	1	2	2	2
WH-10	1	2	2	2
WH-12	2	1	2	2
WH-13	2	1	1	1
WH-14	2	1	2	2
WH-15	2	2	2	1
WH-18	1	1	2	1
WH-19	1	0	2	2
CONTROL	0	0	0	0

Table7.4.1 | showing toxicity score on water hyacinth leaves on application of isolated fungus on day 3,5 and 7 respectively,[toxicity score 2(severe damage) followed by disease score 1(mild damage) and disease score 0(no disease or symptoms)].

7.5 Whole plant bioassay

Out of sixteen isolates, five isolates (WH-2, WH-3, WH-7, WH-9, WH-19) caused severe diseased with almost ≥50% foliar damage when tested in the whole plant pathogenecity test, where as the remaining isolates (WH-1, WH-4, WH-5, WH-6,WH-8, WH-10,WH-12,WH-13, WH-14,WH-15,WH-18) damaged plant leaves around foliar damage percentage < 50%, but did not result in plant death.

WH-1

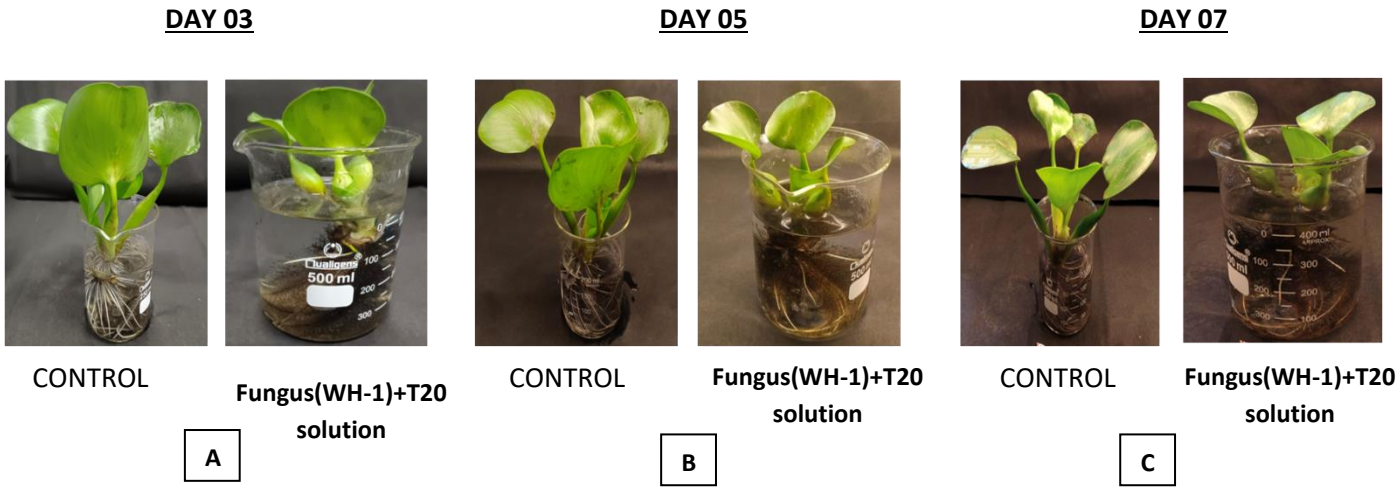


Figure7.5.2 | Whole plant assay of fungus(WH-1) A| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-2

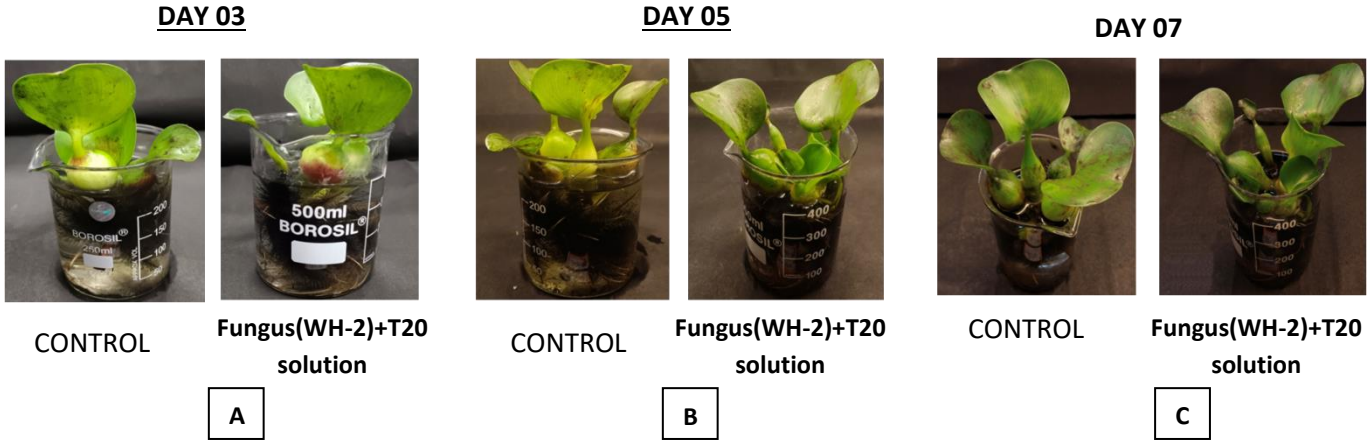


Figure7.5.3 | Whole plant assay of fungus(WH-2) A| application of fungus (WH-2) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-2) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-2) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth oobserved on day 05.

WH-3

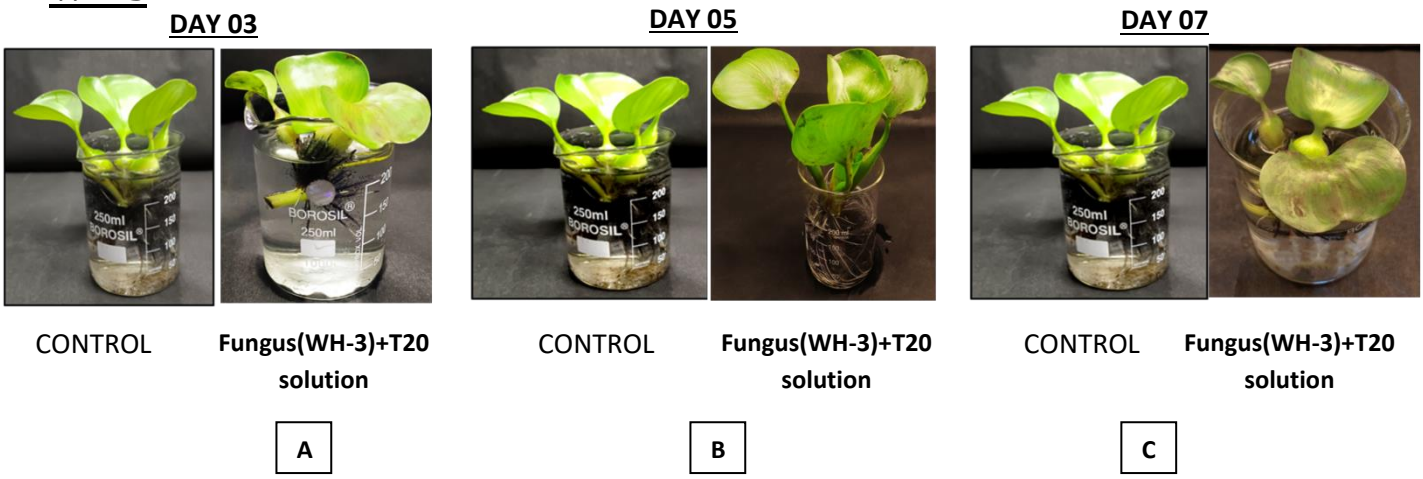


Figure7.5.4 | Whole plant assay of fungus (WH-3) A| application of fungus (WH-3) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-3) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-3) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-4

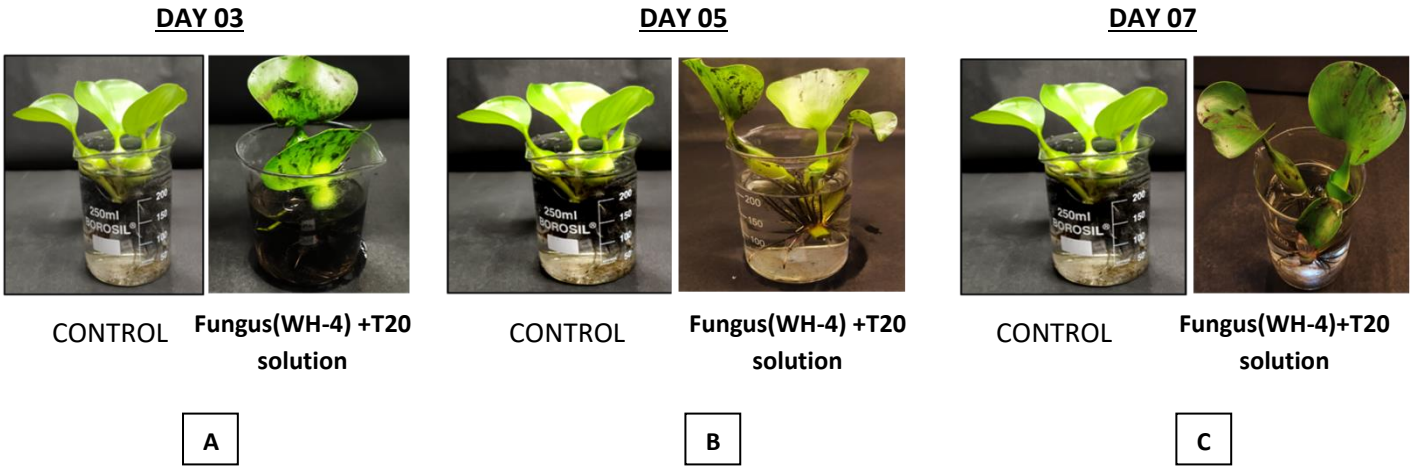


Figure7.5.5 | Whole plant assay of fungus(WH-4) A| application of fungus (WH-4) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-4) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-4) with T20 solution (0.01%) on healthy whole plant Water Hyacinth observed on day 05.

WH-5

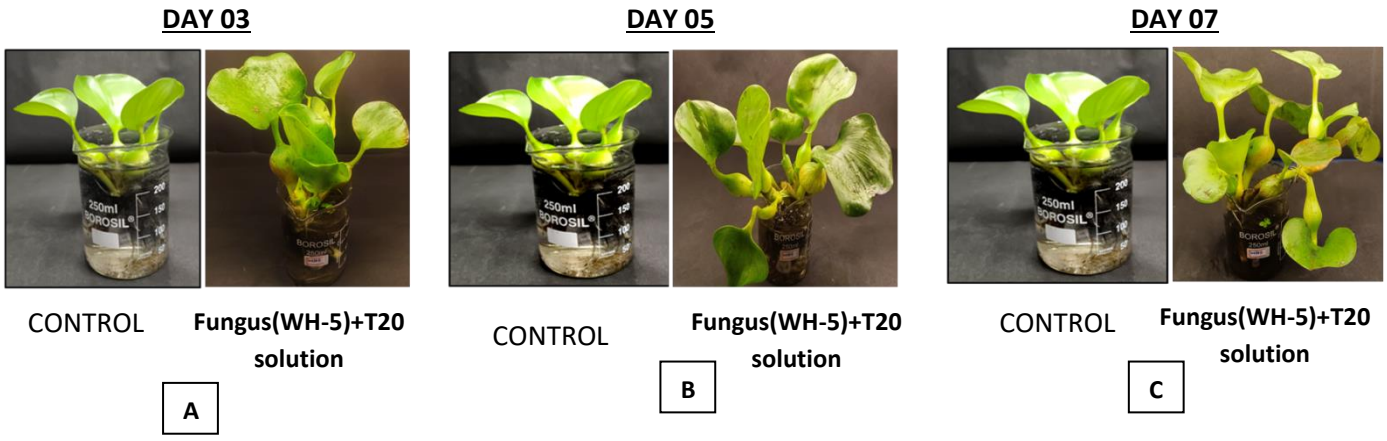


Figure7.5.6 | Whole plant assay of fungus(WH-5) A| application of fungus (WH-5) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-5) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-5) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-6

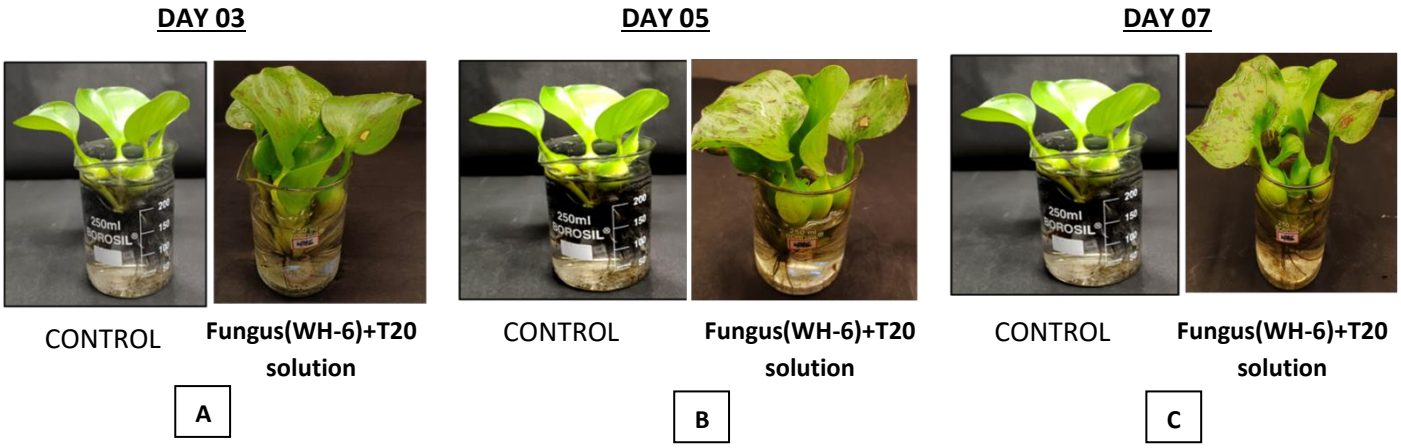


Figure7.5.7 | Whole plant assay of fungus(WH-6) A| application of fungus (WH-6) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-6) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-6) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-7

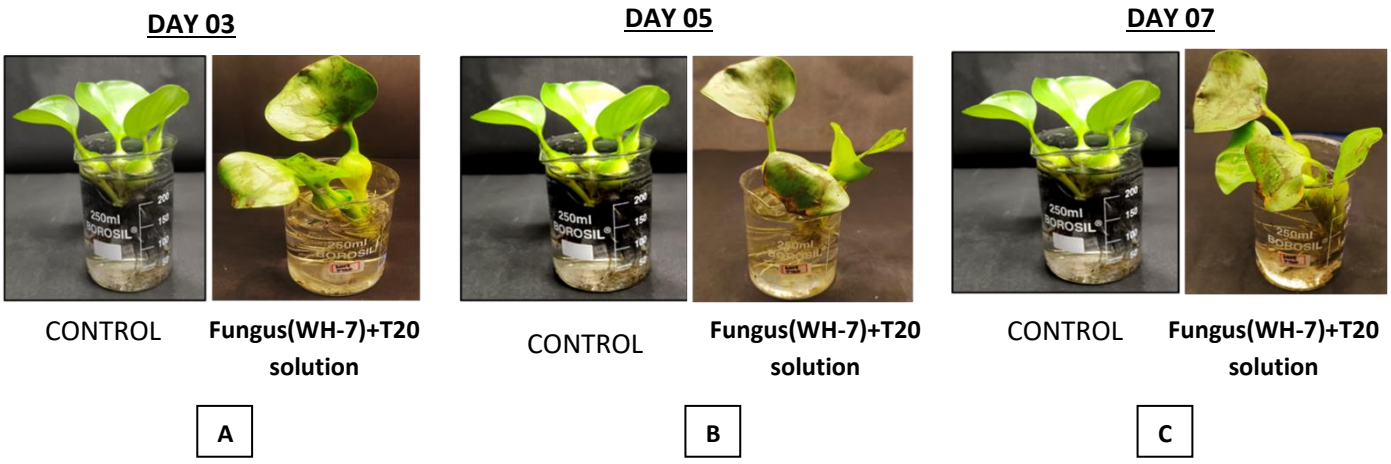


Figure7.5.8 | Whole plant assay of fungus(WH-7) A| application of fungus (WH-7) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-7) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-7) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-8

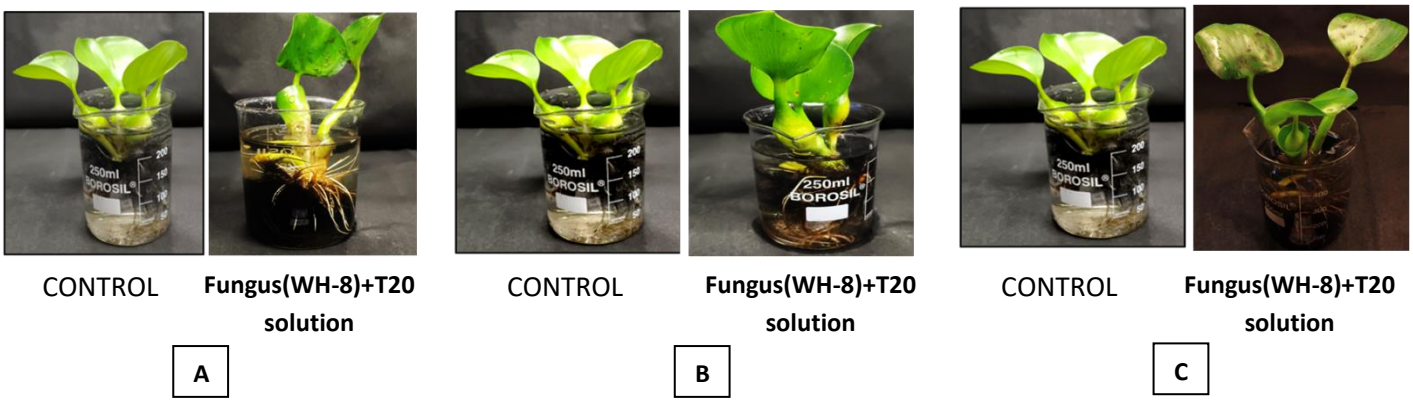


Figure7.5.9 | Whole plant assay of fungus(WH-8) A| application of fungus (WH-8) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-8) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-8) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-9

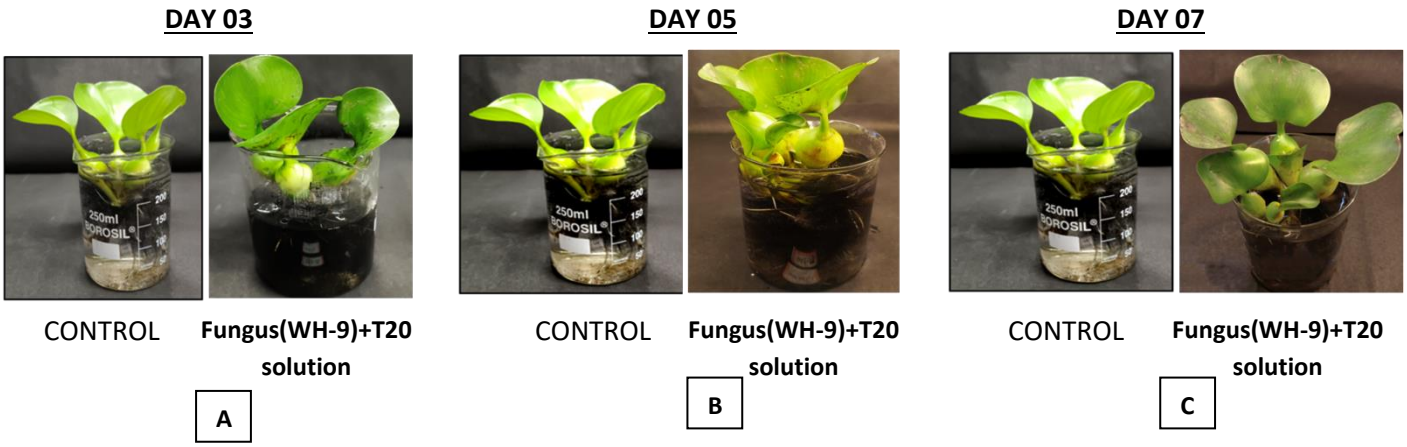


Figure7.5.10 | Whole plant assay of fungus(WH-1) A| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-1) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05

WH-10

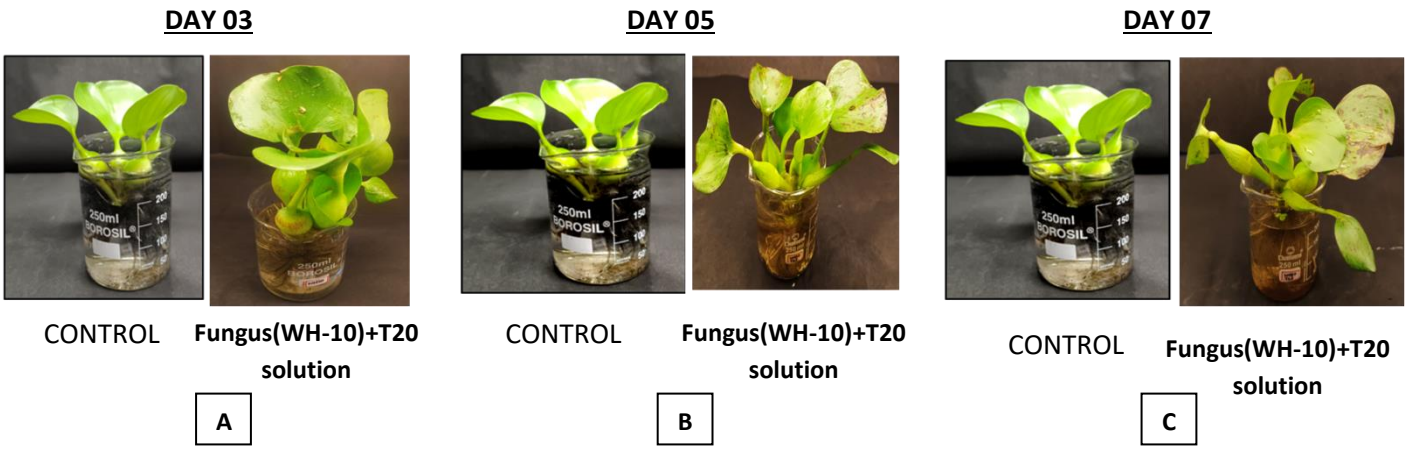


Figure7.5.11 | Whole plant assay of fungus(WH-10) A| application of fungus (WH-10) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-10) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-10) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-12

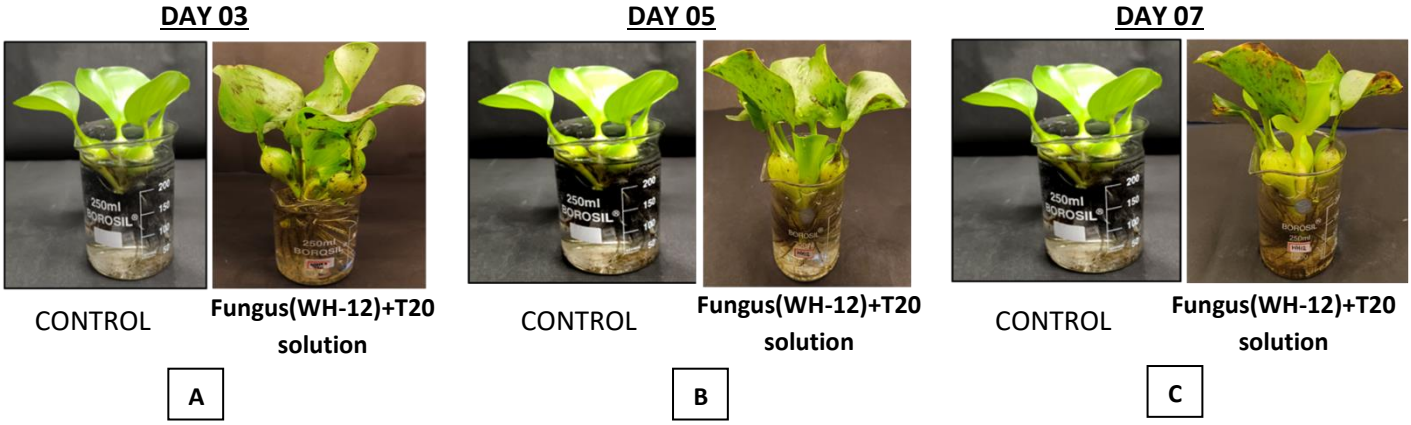


Figure7.5.12 | Whole plant assay of fungus(WH-12) A| application of fungus (WH-12) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-12) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-12) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-13

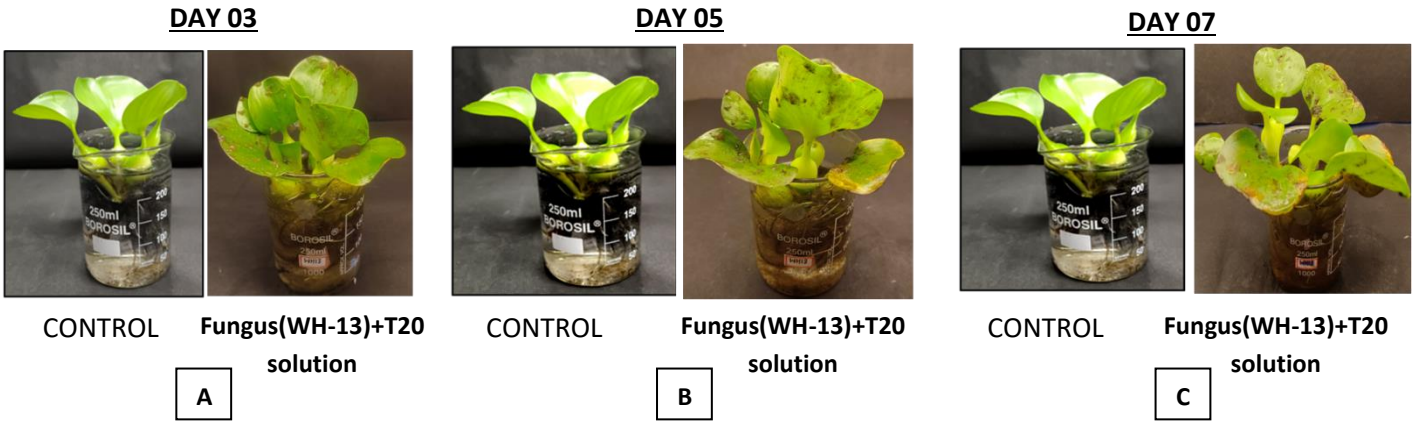


Figure7.5.13 | Whole plant assay of fungus(WH-13) A| application of fungus (WH-13) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-13) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-13) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.

WH-14

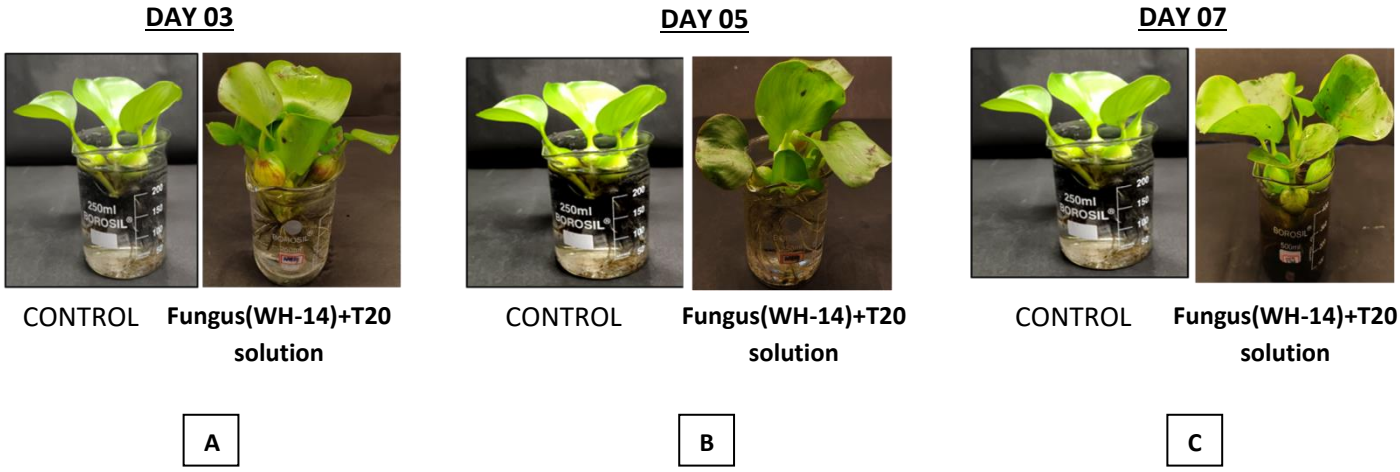


Figure7.5.14 | Whole plant assay of fungus(WH-14) A| application of fungus (WH-14) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-14) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-14) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-15

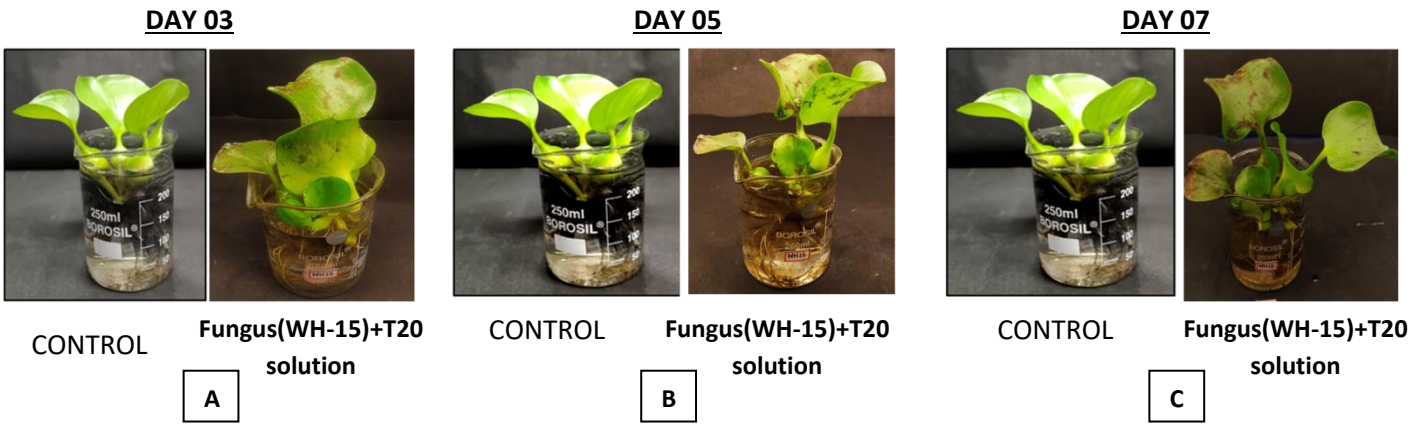


Figure7.5.15 | Whole plant assay of fungus(WH-15) A| application of fungus (WH-15) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 03 B| application of fungus (WH-15) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05 C| application of fungus (WH-15) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth observed on day 05.

WH-18

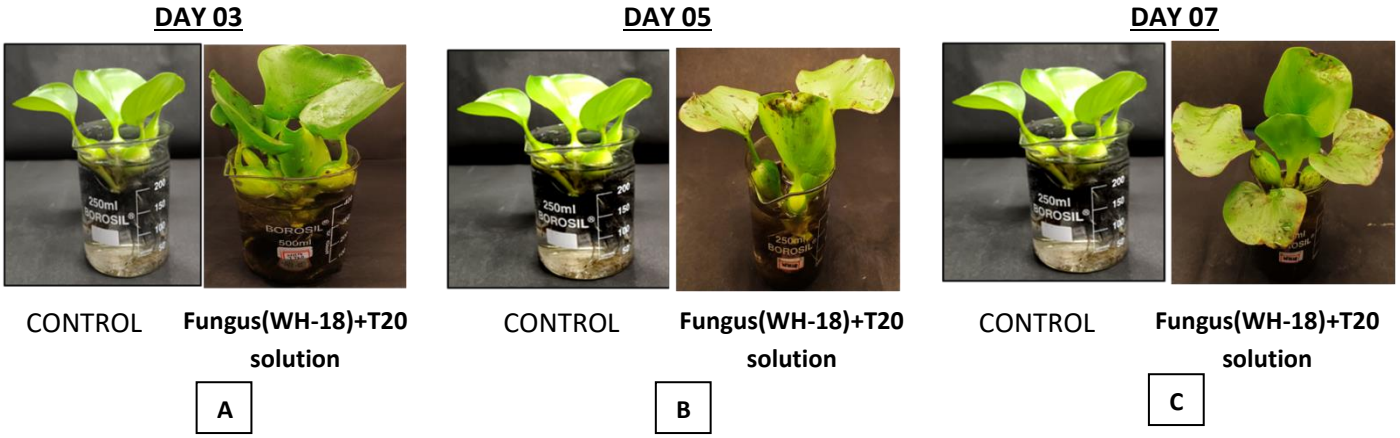


Figure7.5.16 | Whole plant assay of fungus(WH-18) A| application of fungus (WH-18) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-18) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-18) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.

WH-19

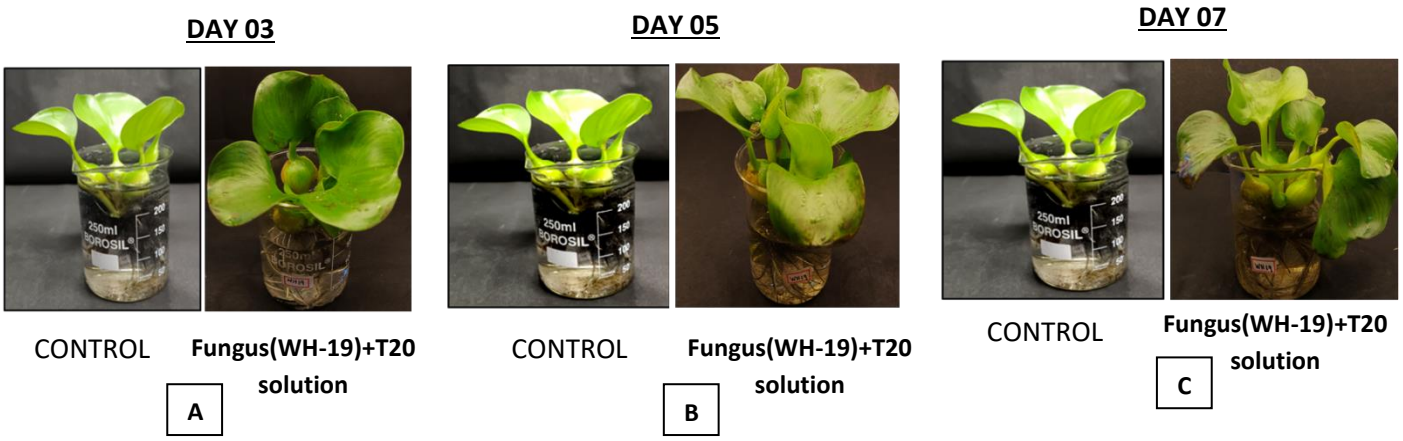


Figure7.5.17 | Whole plant assay of fungus(WH-19) A| application of fungus (WH-19) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 03 B| application of fungus (WH-19) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05 C| application of fungus (WH-19) with T20 solution (0.01%) on healthy whole plant of Water Hyacinth of observed on day 05.

	DAY-03	DAY-05	DAY-07
STRAINS	**MEAN (FD%) ±SE**	**MEAN (FD%) ±SE**	**MEAN (FD%) ±SE**
CONTROL	**0**	**0**	**0**
WH-1	**12.5±12.5**	**22.5±2.5**	**42.5±17.5**
WH-2	**0**	**35±15**	**57.5±17.5**
WH-3	**16.6667±16.6667**	**16.6667±16.6667**	**66.6667±33.3333**
WH-4	**0**	**16.6667±16.6667**	**29.1667±4.16667**
WH-5	**0**	**16.6667±16.6667**	**45.8333±20.8333**
WH-6	**0**	**40±20**	**40±20**
WH-7	**0**	**37.5±12.5**	**62.5±12.5**
WH-8	**0**	**45.8333±20.8333**	**45.8333±20.83333**
WH-9	**25±0**	**37.5±12.5**	**50±25**
WH-10	**10±10**	**26.6667±26.6667**	**45±5**
WH-12	**6.25±6.25**	**19.6429±5.3714**	**33.0357±4.46429**
WH-13	**5.55556±5.55556**	**21.1111±21.1111**	**21.1111±1.11111**
WH-14	**0**	**8.3333±8.3333**	**18.3333±1.66667**
WH-15	**0**	**33.3333±16.6667**	**33.3333±16.6667**
WH-18	**0**	**8.33333±8.33333**	**18.3333±1.66667**
WH-19	**12.5±12.5**	**37.5±12.5**	**50±0**

Table 7.5.1 | FD(%) of whole plant bioassay of fungal strains observed on day 3, 5 and 7 respectively.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
FD (%) * STRAIN	Between Groups	(Combined)	1791.992	15	119.466	1.257	.327
	Within Groups		1520.409	16	95.026		
	Total		3312.401	31			

Table 7.5.2 | ANOVA table obtained by statistical analysis (SPSS) showing no significant difference between fungal strains on day 3 of whole plant bioassay.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
FD (%) * STRAIN	Between Groups	(Combined)	4160.329	15	277.355	.776	.686
	Within Groups		5716.812	16	357.301		
	Total		9877.141	31			

Table 7.5.3 | ANOVA table obtained by statistical analysis (SPSS) showing no significant difference between fungal strains on day 3 of whole plant bioassay.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
FD (%) * STRAIN	Between Groups	(Combined)	6611.980	15	440.799	.856	.616
	Within Groups		8239.551	16	514.972		
	Total		14851.531	31			

Table 7.5.4| ANOVA table obtained by statistical analysis (SPSS) showing no significant difference between fungal strains on day 3 of whole plant bioassay.

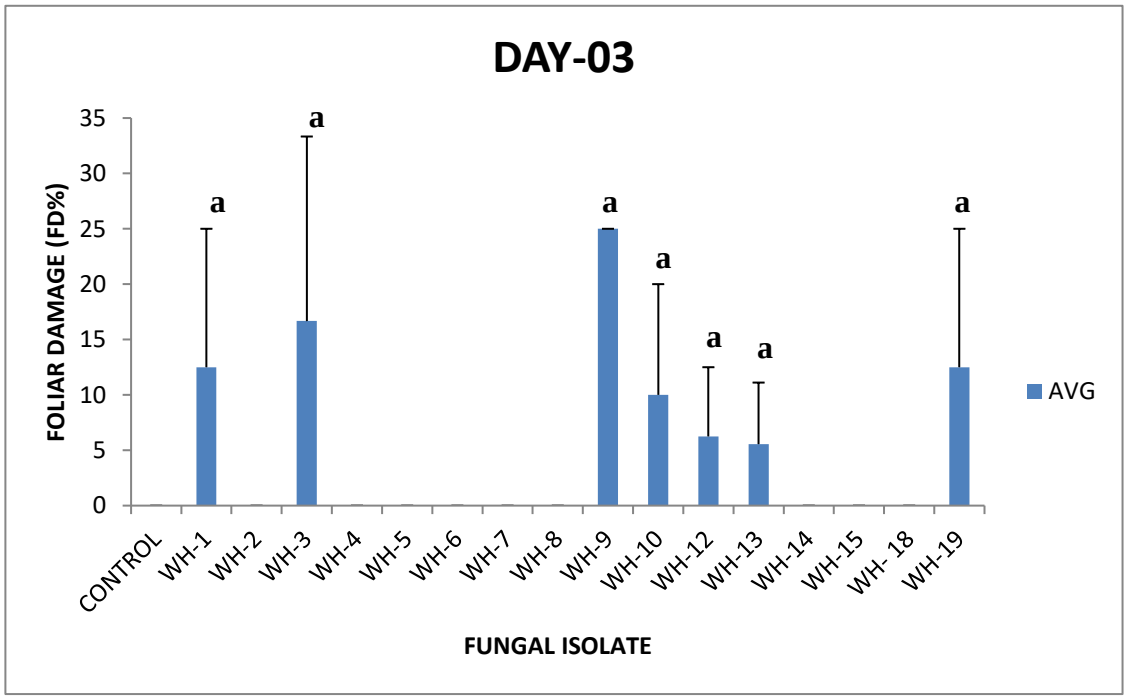


Figure 7.5.18 |Effect of different fungal isolates on water hyacinth after 3 days of inoculation Vertical bars represent the standard error of the mean. Means marked with the same letter(s) above the bars(a) are not significantly different according to Bonferroni's tests (P<0.05).

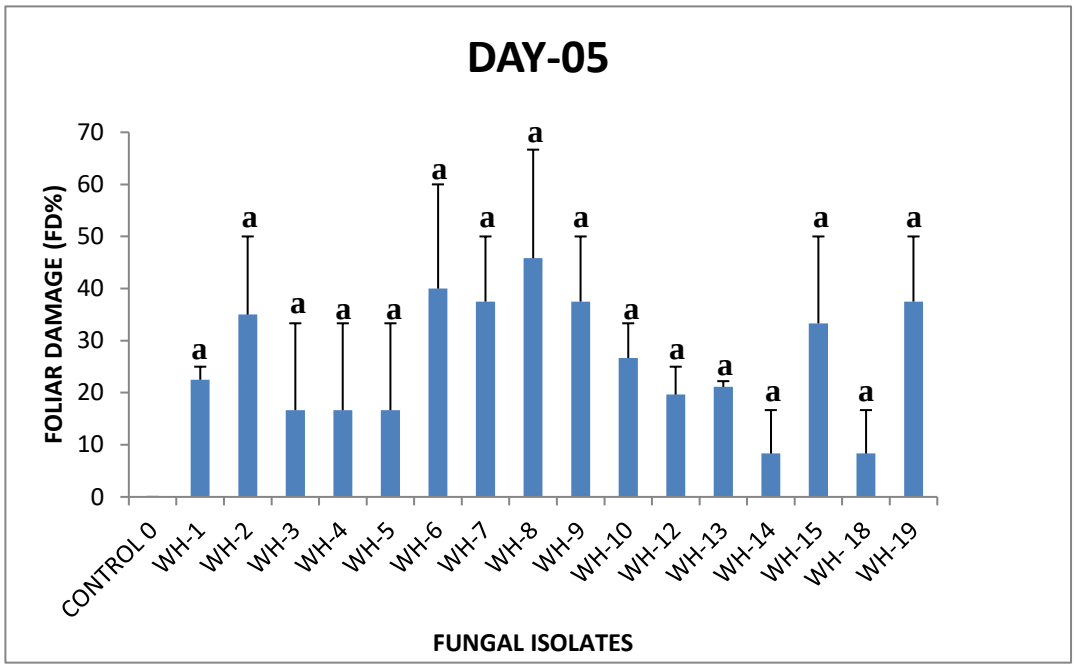


Figure 7.5.19 |Effect of different fungal isolates on water hyacinth after 5 days of inoculation Vertical bars represent the standard error of the mean. Means marked with the same letter(s) above the bars(a) are not significantly different according to Bonferroni's tests (P<0.05).

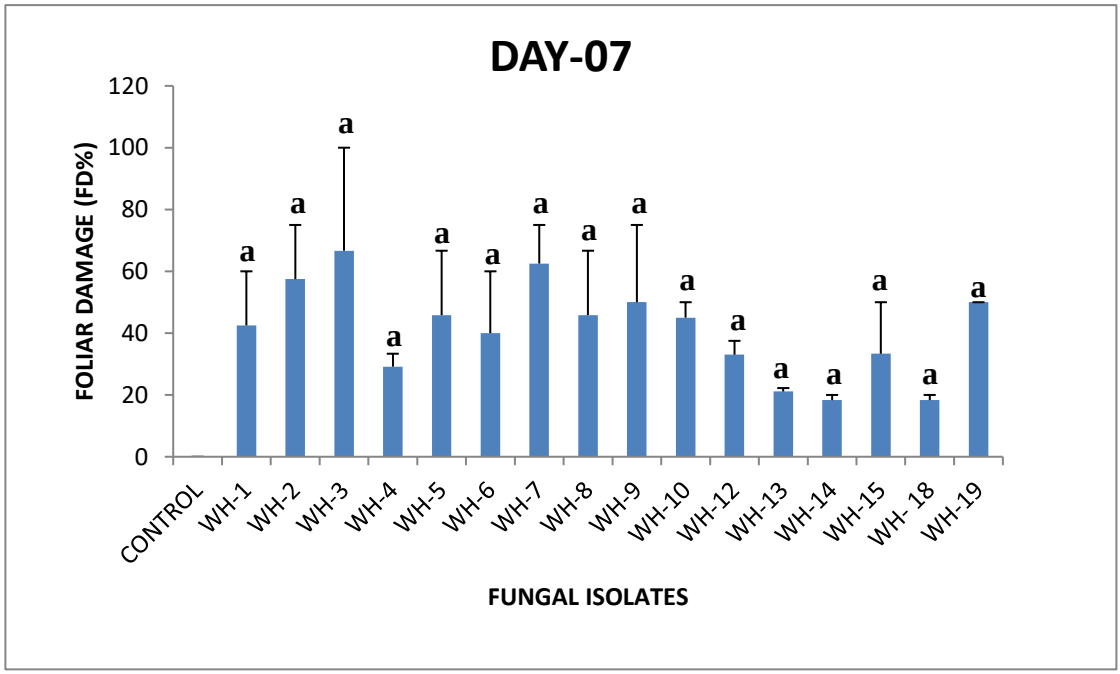
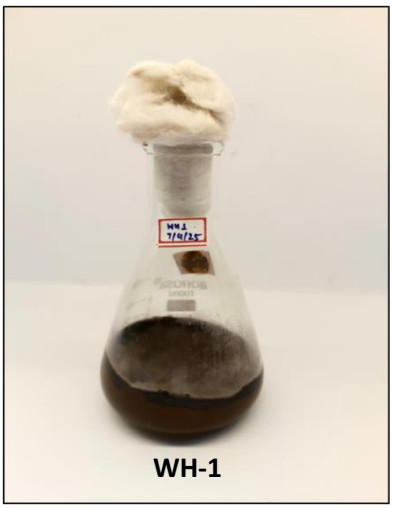


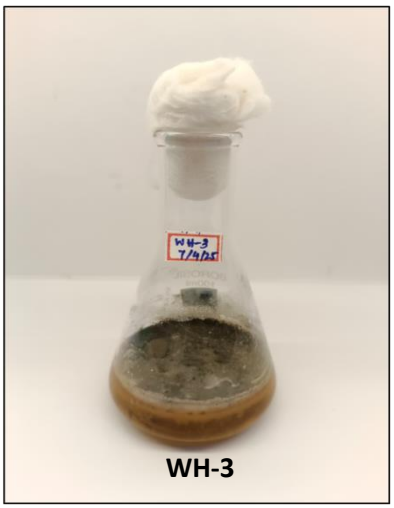
Figure 7.5.20 |Effect of different fungal isolates on water hyacinth after 7 days of inoculation Vertical bars represent the standard error of the mean. Means marked with the same letter(s) above the bars(a) are not significantly different according to Bonferroni's tests (P<0.05).

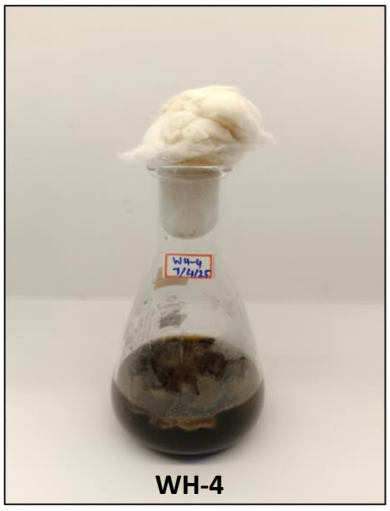
7.6 Extraction crude metabolites

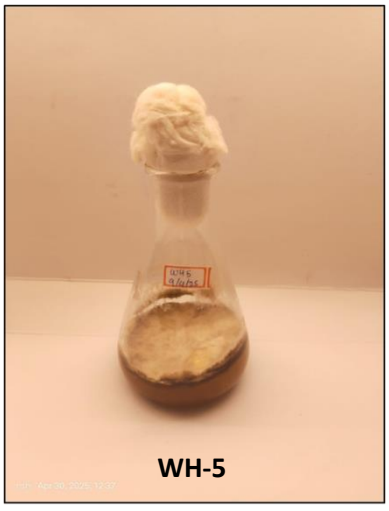
From the 21 days incubated fungal cultures of strains (WH-1,WH-2,WH-3, WH-4, WH-5, WH-6, WH-7,WH-8,WH-9,WH-10,WH-12,WH-13, WH-14,WH-15,WH-18,WH-19) lab accession numbers were successfully extracted to get the crude metabolites from this fungal strains of water hyacinth.


WH-1

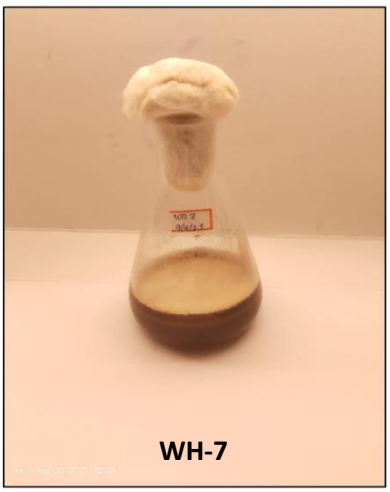
WH-2

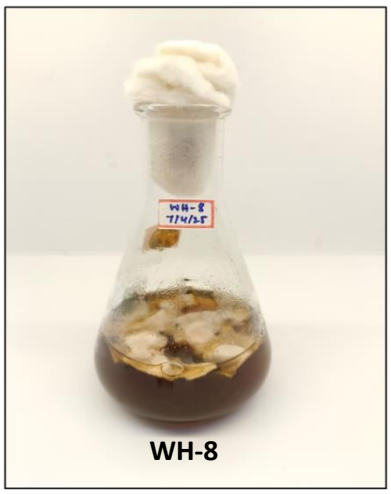

WH-3

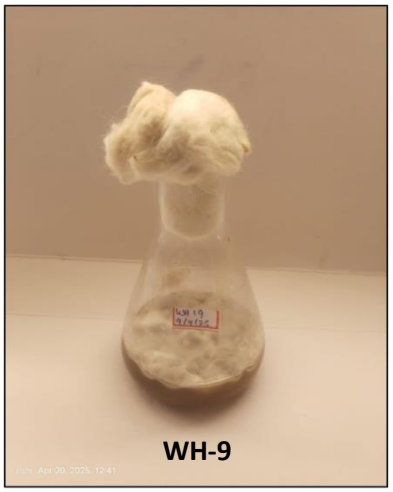

WH-4

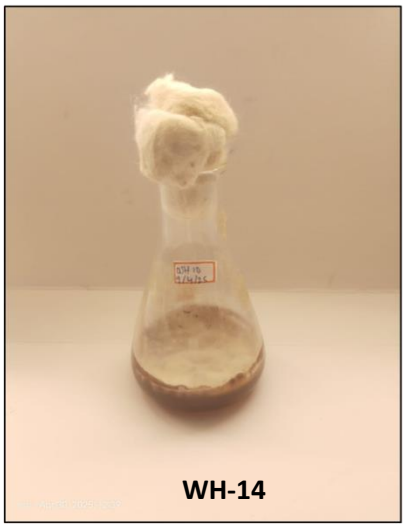

WH-5

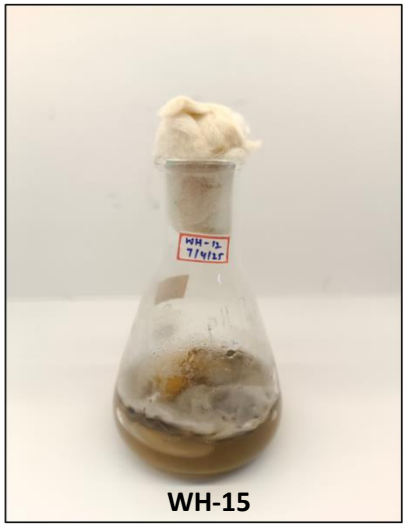
WH-6


WH-7


WH-8


WH-9





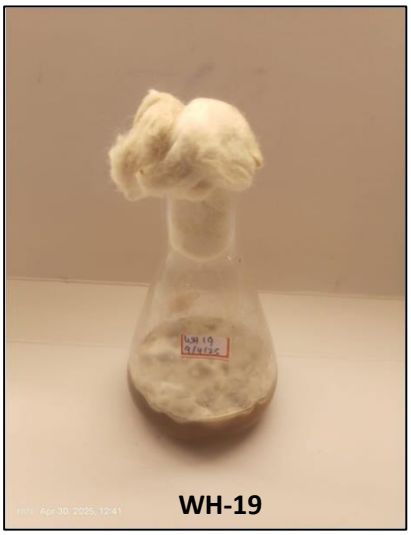


Figure7.6.1| Fungal metabolites strains of (WH-1,WH-2,WH-3, WH-4, WH-5, WH-6, WH-7, WH-8,WH-9,WH-10,WH-12,WH-13, WH-14,WH-15,WH-18,WH-19) lab accession numbers on Potato Dextrose Broth

7.7 Toxicity Test of Crude Metabolite

Five isolates (WH-2, WH-3, WH-7, WH-9, WH-19) that showed high levels of pathogenicity in whole plant assay i.e., (≥50% foliar damage) were tested in the metabolite assay, to evaluate the toxic effects of crude metabolites on a selective biological system. Control with negligible symptoms was observed.

WH-2

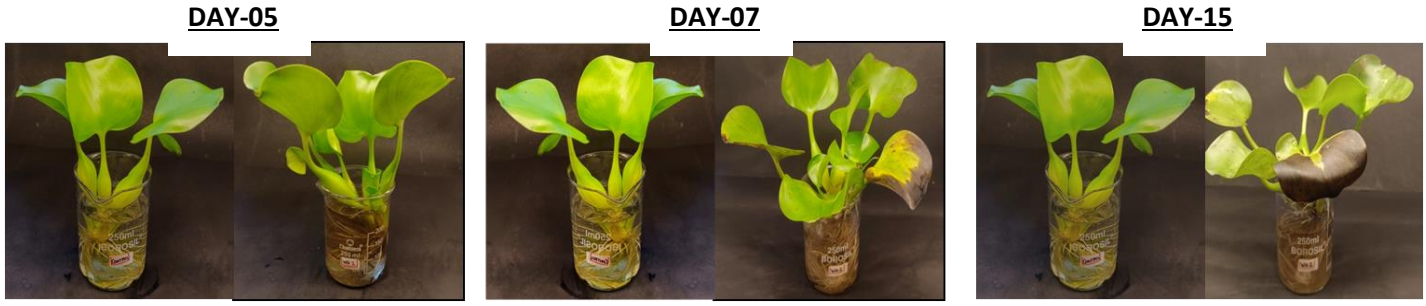


Figure7.7.2| Application of metabolite spray (WH-2) on the whole plant of water hyacinth of observed on day 03, 05 and 07 respectively.

WH-3

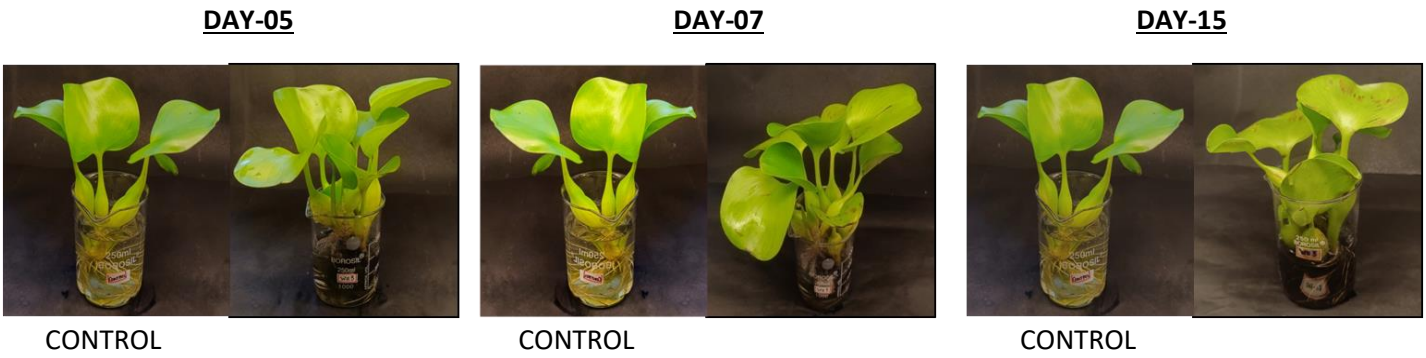


Figure7.7.3| Application of metabolite spray (WH-3) on the whole plant of water hyacinth of) observed on day 03, 05 and 07 respectively.

WH-7

DAY-05 **DAY-07** **DAY-15**

CONTROL CONTROL CONTROL

Figure7.7.4| Application of metabolite spray (WH-7) on the whole plant of water hyacinth of observed on day 03, 05 and 07 respectively.

WH-9

DAY-05 **DAY-07** **DAY-15**

CONTROL CONTROL CONTROL

Figure7.7.5| Application of metabolite spray (WH-9) on the whole plant of water hyacinth of observed on day 03, 05 and 07 respectively.

WH-19

DAY-05 **DAY-07** **DAY-15**

CONTROL CONTROL CONTROL

Figure7.7.6| Application of metabolite spray (WH-19) on the whole plant of water hyacinth of observed on day 03, 05 and 07 respectively.

DAY-15	
STRAINS	**MEAN (FD%) ±SE**
WH-2	100±**0**
WH-3	66.07143±**8.928571**
WH-7	70.2381±**13.09524**
WH-9	91.66667±**8.333333**
WH-19	73.333333±**6.666667**
CONTROL	0

Table 7.7.1 | (FD%) of toxicity test of crude metabolites of day 15

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
FD(%) * STRAINS	Between Groups	(Combined)	3848.390	4	962.098	22.677	.002
	Within Groups		212.132	5	42.426		
	Total		4060.522	9			

Table 7.7.2 | ANOVA table obtained by statistical analysis (SPSS) showing significant difference between fungal strains

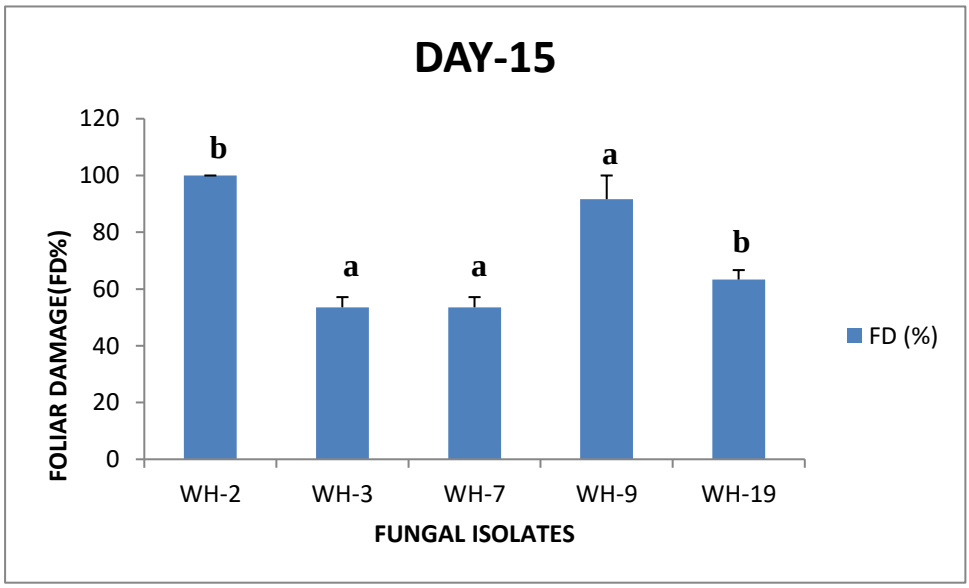


Figure 7.7.7 |Effect of different fungal isolates on water hyacinth after 15 days of inoculation Vertical bars represent the standard error of the mean. Means marked with the same letter(s) above the bars are not significantly different according to Bonferroni's tests (P<0.05).

7.8 Morphological Identification

The two fungal strains, WH-2 and WH-9, which exhibited most significant toxicity against water hyacinth, were examined under a microscope for morphological identification, and was identified as *Curvularia* spp.

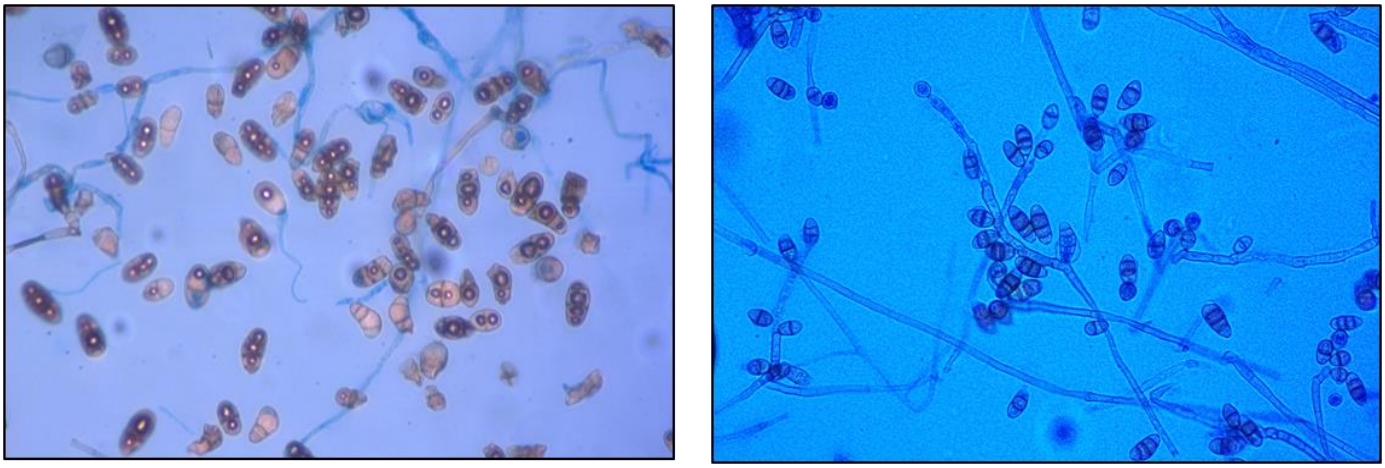


Figure7.8.1 | Morphological characteristics of *Curvularia* species showing typical colony appearances and spore structures under microscope.

CHAPTER 08: SIGNIFICANCE OF THE STUDY

1. Shedding Light on Biocontrol Agents and the Potential for Bioherbicides

The study aims to uncover and evaluate effective biocontrol agents for managing the invasive water hyacinth (*Pontederia crassipes*), with a focus on fungal pathogens capable of producing bioactive metabolites. These fungal agents, when properly harnessed, provide an innovative and sustainable solution for combating water hyacinth infestations. By isolating and studying fungal species that exhibit pathogenicity toward this invasive plant, the study seeks to develop bioherbicides that can be effectively deployed under real-world field conditions. This approach not only addresses the ecological and economic challenges posed by water hyacinth but also provides a pathway to integrated weed management strategies. The development of bioherbicides aligns with global sustainability goals by offering an alternative that is both effective and environmentally responsible (Barreto & Evans, 1996; Azevedo et al., 2020).

2. Replacing Chemical Herbicides with Fungal Bioherbicides

The reliance on chemical herbicides for invasive weed management has raised concerns about environmental degradation, non-target effects, and the emergence of herbicide-resistant weed populations. Fungal bioherbicides, derived from the secondary metabolites of phytopathogenic fungi, present a promising alternative. These bioherbicides:

- **Target Specificity**: Selectively impact water hyacinth, minimizing harm to native flora and fauna (Charudattan et al., 2001).
- **Eco-Friendly Decomposition**: Degrade naturally without leaving harmful residues in the environment (Auld et al., 1993).
- **Reduction of Chemical Load**: Decrease the usage of synthetic herbicides, thereby mitigating associated risks to human and ecological health (Evans & Reeder et al., 2001).

By integrating fungal bioherbicides into weed management programs, the increasing population of invasive species like water hyacinth can be controlled in a manner that safeguards biodiversity and promotes ecological balance (TeBeest et al., 1992).

3. Identification of Promising Fungal Species with Bioactive Metabolites

A central goal of the study is the identification and characterization of fungal species that produce bioactive molecules with potent herbicidal properties.

Isolation and Screening: Identifying fungi associated with diseased or declining water hyacinth plants and evaluating their ability to produce bioactive metabolites that suppress plant growth (McFadyen et al., 1998).

The identification of the most promising fungal species will not only facilitate the immediate development of bioherbicides but also contribute to a broader understanding of fungal bioactive molecules. This knowledge can drive further research into novel applications of these metabolites in agriculture, environmental management, and biotechnology (Boyette et al., 2008).

CHAPTER 09: FUTURE PROSPECTS

The isolation and preliminary identification of phytopathogenic fungi with bioherbicidal potential against *Pontederia crassipes* (water hyacinth) lay a promising foundation for the development of eco-friendly, biological control strategies. While chemical herbicides are effective, their environmental and ecological impacts necessitate the exploration of sustainable alternatives such as mycoherbicides.The isolation of phytopathogenic fungi with potential bioherbicidal activity against *Pontederia crassipes* (water hyacinth) represents a significant step towards the development of environmentally responsible weed control strategies. As chemical herbicides continue to raise concerns due to their ecological impact and resistance development in weeds, bioherbicides derived from pathogenic fungi offer a promising alternative (Charudattan, 2001; Hoagland, 2001). Investigating the production of phytotoxic metabolites and enzymes by fungal isolates could lead to the discovery of novel compounds with herbicidal properties. Techniques such as HPLC and GC-MS can be employed for metabolite profiling (Vurro et al., 2001). Before large-scale application, the environmental safety of fungal bioherbicides must be assessed, including their effect on aquatic biodiversity, soil microbiota, and water quality (Harding & Raizada, 2015). In conclusion, the future of fungal bioherbicides is promising but depends on multidisciplinary efforts involving taxonomy, biotechnology, formulation science, ecology, and policy. Advancing this research could contribute meaningfully to sustainable management of invasive aquatic weeds such as water hyacinth.

---

1. Host Range Studies for Ecological Safety

Before deploying any biocontrol agent, it is essential to conduct detailed host range studies. These studies assess the potential impacts of the agent on non-target species, ensuring it specifically targets the intended pest without causing harm to other organisms. Of particular concern are native plant species and those of economic significance, as these are vital components of ecosystems and agricultural systems. Any negative impact on such plants could disrupt local biodiversity, economic stability, and ecological balance (McFadyen et a.,1998; Wapshere.,1974). Comprehensive host range assessments typically involve laboratory, greenhouse, and field tests to simulate various environmental conditions and interactions (Blossey, 1999). By thoroughly understanding the potential risks, researchers can make informed decisions and develop mitigation strategies if necessary (Sheppard et al., 2003).

2. Molecular Identification for Taxonomic Accuracy

The accurate identification of a fungal biocontrol agent is a critical step in its development and application. Molecular identification techniques, such as polymerase chain reaction (PCR)-based DNA barcoding and genome sequencing, provide reliable means of determining the exact species and strain of the fungus (Hebert et al., 2003; Schoch et al., 2012). This precision is essential not only for confirming the agent's identity but also for avoiding the accidental use of closely related, potentially harmful species (Seifert et al., 2007). Additionally, proper identification supports compliance with regulatory standards, facilitates reproducibility in research, and contributes to a deeper understanding of the organism's biology (Rossman et al., 2006). The integration of molecular tools ensures that the selected fungal agent is both safe and effective for the intended purpose.

CHAPTER 10 : CONCLUSION

The fungal isolates WH-2 and WH-9, identified as *Curvularia* spp., have been observed to cause significant disease symptoms in *Pontederia crassipes* (water hyacinth) across various regions, including India. Field surveys conducted in West Bengal between 2017 and 2023 documented severe infections, with some fungal isolates inducing over 90% disease severity in the target weed (Mukherjee et al., 2023; Tiwari et al., 2020). This widespread occurrence suggests that the fungus may be globally distributed, potentially spreading alongside its invasive host (*Barreto & Evans, 1996*).

Preliminary studies have indicated that crude metabolites produced by *Curvularia* spp. exhibit phytotoxic effects on *Pontederia crassipes* (water hyacinth), highlighting their potential as bioherbicidal agents (Boyette et al., 2008). However, to advance towards commercial application, comprehensive long-term studies are essential. These investigations should focus on assessing the stability of these metabolites, their efficacy under diverse field conditions, and their overall viability as practical bioherbicides (Evans, 2000). Such research will be crucial in determining the feasibility of integrating *Curvularia* spp. and its metabolites into sustainable weed management strategies (Charudattan, 2001).

CHAPTER 11 : REFERENCES

Auld, B. A., Morin, L., & Brown, J. F. (2003). Host specificity testing of pathogens for biological control of weeds: a scientific approach. Australian Centre for International Agricultural Research.

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. (1996). **Introductory Mycology**. Wiley.

Agrios, G. N. (2005). **Plant Pathology**. Elsevier Academic Press.

Atlas, R. M., & Snyder, J. W. (2006). **Handbook of Media for Clinical and Public Health Microbiology**. CRC Press.

Auld, B. A. (1993). Opportunities for bioherbicide development. *Plant Protection Quarterly*, *8*(4), 150–154.

Azevedo, J. L., Maccheroni, W., Pereira, J. O., & de Araújo, W. L. (2020). Endophytic microorganisms: A review on insect control and recent advances on tropical plants. *Acta Scientiarum Biological Sciences, 42*(2), 67–80.

Babu, R. M., Sajeena, A., Seetharaman, K., & Velazhahan, R. (2003). Bioassay of Phytotoxins Produced by *Alternaria alternata* in the Development of a Bioherbicide for Water Hyacinth Control. *Weed Biology and Management, 3*(4), 223–227.

Bailey, K. L., Boyetchko, S. M., & Längle, T. (2010). Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the adoption of microbial pesticides. *Biological Control, 52*(3), 221–229.

Baldrick, P. (2005). Toxicology testing strategies for the 21st century. *Toxicology, 206*(2), 179–190.

Barreto, R. W., & Evans, H. C. (1996). The mycobiota of the water hyacinth in the upper Amazon basin with particular reference to fungal pathogens for biological control. *Mycological Research, 100*(5), 541–546.

Baldrick, P. (2005). Toxicology testing strategies for the 21st century. *Toxicology, 206*(2), 179–190.

Barnett, H. L., & Hunter, B. B. (1998). **Illustrated Genera of Imperfect Fungi**. APS Press.

Barreto, R. W., & Evans, H. C. (1996). Fungal pathogens of some Brazilian aquatic weeds and their potential use as biocontrol agents. *Mycological Research, 100*(6), 598–606.

Barreto, R. W., & Evans, H. C. (1996). Fungal pathogens of some Brazilian aquatic weeds and their potential use in biocontrol. *Weed Research, 36*(4), 467–474.

Benson, D. M., & Baker, K. F. (1974). "Sterilization Techniques for Plant Pathology". *Annual Review of Phytopathology*.

Blossey, B. (1999). Before, during and after: The need for long-term monitoring in invasive plant species management. *Biological Invasions, 1*(3), 301–311.

Boyette, C. D., Hoagland, R. E., & Weaver, M. A. (2008). Interaction of a fungal pathogen with glyphosate for controlling invasive plant species. *Weed Biology and Management, 8*(3), 134–142.

Campbell, C. L., & Madden, L. V. (1990). **Introduction to Plant Disease Epidemiology**. Wiley-Blackwell.

Chandramohan, S., & Charudattan, R. (2001). "Fungi as biocontrol agents for invasive weeds." *Mycological Research.*

Charudattan, R. (2001). Biological control of water hyacinth by using pathogens: Opportunities, challenges, and recent developments. *Weed Management Society of Japan, 46*(4), 129–136.

Cairns, T. C., Nai, C., & Meyer, V. (2018). How a fungus shapes biotechnology: 100 years of *Aspergillus niger* research. *Fungal Biology and Biotechnology*, *5*(1), 1–14.

Center, T. D., Hill, M. P., Cordo, H., & Julien, M. H. (2002). Water Hyacinth. In *Biological Control of Invasive Plants in the United States* (pp. 41–64). USDA Agricultural Research Service.

Charudattan, R. (2001). Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. *BioControl, 46*, 229–260.

Charudattan, R. (2001). Biological Control of Water Hyacinth by Using Pathogens: Opportunities, Challenges, and Recent Developments. In *Biological and Integrated Control of Water Hyacinth, Eichhornia crassipes*. ACIAR Proceedings.

Charudattan, R. (2005). Ecological, practical, and political inputs into selection of weed targets: What makes a good biological control target? *Biological Control, 35*(3), 183–196.

Charudattan, R. (2001). "Biological Control of Water Hyacinth by Pathogens: Opportunities, Challenges, and Recent Developments". *Biocontrol News and Information*, 22(1), 35-46.

Center, T.D., Spencer, N.R., & Cofrancesco, A.F. (1989). "Invasive Plant Management and Eradication in the United States". *Environmental Management*.

Cilliers, C. J., Hill, M. P., Ogwang, J. A., & Ajuonu, O. (2003). Aquatic weeds in Africa and their control. *Proceedings of the African Water Weeds Workshop*, 25–27.

Cos, P., Vlietinck, A. J., Berghe, D. V., & Maes, L. (2006). Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology, 106*(3), 290–302.

Cronin, M. T., & Madden, J. C. (2010). In silico toxicology: Principles and applications. Royal Society of Chemistry.

Coetzee, J.A., Hill, M.P., & Schlange, D. (2009). "Biological Control of Water Hyacinth: An Evaluation of Efficacy and Impact". *African Entomology*, 17(2), 469-480.

Daigle, D. J., Connick, W. J., & Quimby, P. C. (1999). Formulation of bioherbicides using a dry alginate matrix. *Weed Technology, 13*(4), 626–632.

Ekwall, B. (1999). Overview of toxicity tests for the safety evaluation of bioactive substances. *Toxicology in Vitro, 13*(4), 665–673.

Eloff, J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology, 60*(1), 1–8.

Evans, H. C., & Reeder, R. H. (2001). Fungal pathogens of weeds. In *Biological Control: Measures of Success* (pp. 17–37). Springer..Gopal, B. (1987). Water Hyacinth. Elsevier Science Publishers.

Gopal, B., & Sharma, K. P. (1981). Water Hyacinth (*Eichhornia crassipes*): The Most Troublesome Weed of the World. Hindasia Publishers, New Delhi.

Gomez, K. A., & Gomez, A. A. (1984). **Statistical Procedures for Agricultural Research**. John Wiley & Sons.

Gupta, R., & Sharma, V. (2019). "Aseptic techniques in mycology." *Laboratory Mycology Guide.*

Harding, D. P., & Raizada, M. N. (2015). Controlling weeds with fungi, bacteria and viruses: A review. *Frontiers in Plant Science, 6,* 659.

Harvey, A. L., Edrada-Ebel, R., & Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews Drug Discovery, 14*(2), 111–129.

Harrigan, W.F. (1998). *Laboratory Methods in Food Microbiology*. Academic Press.

Harman, G. E., & Kubicek, C. P. (1998). **Trichoderma and Gliocladium**. CRC Press.

Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313–321.

Hoagland, R. E. (2001). Microbial allelochemicals and pathogens as bioherbicidal agents. *Weed Technology, 15*(4), 835–857.

Julien, M. H., Hill, M. P., Tipping, P. W., & Center, T. D. (1999). Biological control of water hyacinth. *International Organization for Biological Control Bulletin, 22*, 8–20.

Julien, M. H., Hill, M. P., Tipping, P. W., & Center, T. D. (2001). Biological Control of Water Hyacinth under Conditions of Maintenance Management,

Julien, M.H., & Orapa, W. (1999). "Biological Control of Water Hyacinth". *Biocontrol News and Information*.

Jiang, H., Zhang, X., & Zhang, Y. (2015). Role of fungal metabolites in bioherbicide development. *Current Biotechnology, 4*(2), 112–118.

Kumar, A., et al. (2018). "Media preparation for fungal studies." *Biotechnology Techniques.*

Larone, D. H. (2011). **Medically Important Fungi: A Guide to Identification**. ASM Press.

Meyer, V., Basenko, E. Y., Benz, J. P., et al. (2016). Growing a circular economy with fungal biotechnology: a white paper. *Fungal Biology and Biotechnology, 3*(1), 1–23.

McFadyen, R. E. (1998). Biological control of weeds. *Annual Review of Entomology, 43*(1), 369–393.

Mishra, S., Tiwari, S., & Kumar, P. (2020). Bioherbicides: Techniques for preparation and application of fungal pathogens. *Indian Journal of Plant Protection, 48*(1), 87–93.

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products, 83*(3), 770–803.

Papagianni, M. (2007). Advances in citric acid fermentation by *Aspergillus niger*: Biochemical aspects, membrane transport, and modeling. *Biotechnology Advances, 25*(3), 244–263.

Patel, S. (2012). Threats, management and envisaged utilizations of aquatic weed *Eichhornia crassipes*: An overview. *Reviews in Environmental Science and Bio/Technology, 11*(3), 249–259.

Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993). *Microbiology: Concepts and Applications*. McGraw-Hill Education.

Penfound, W.T., & Earle, T.T. (1948). "The Biology of the Water Hyacinth". *Ecological Monographs*.

Pieterse, A.H., & Murphy, K.J. (1990). *Aquatic Weeds: The Ecology and Management of Nuisance Aquatic Vegetation*. Oxford University Press.

Pitt, J. I., & Hocking, A. D. (2009). **Fungi and Food Spoilage**. Springer Science & Business Media.

Pointing, S. B. (2001). Feasibility of bioremediation by white-rot fungi. *Applied Microbiology and Biotechnology, 57*(1–2), 20–33.

Reddy, K.R., & DeBusk, W.F. (1985). "Nutrient Removal Potential of Water Hyacinth". *Journal of Environmental Quality*.

Reddy, K.R., & DeBusk, W.F. (1984). "Nutrient Removal Potential of Water Hyacinth". *Journal of Environmental Quality*, 13(4), 495-500.

Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., & Andersen, B. (2010). **Food and Indoor Fungi**. CBS-KNAW Fungal Biodiversity Centre.

Schoch, C. L., et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences*, *109*(16), 6241–6246.

Shabana, Y. M., Charudattan, R., & Elwakil, M. A. (2010). Identification, pathogenicity, and safety of a *Myrothecium roridum* isolate as a bioherbicide agent for water hyacinth control. *Biological Control, 54*(2), 94–99.

Shimizu, S. (2016). Microbial production of biofuels and biochemicals from biomass. *Springer Handbook of Biofuels Production*, 151–181.

Singh, R. S., Singh, T., & Pandey, A. (2021). Microbial enzymes in bioconversions of renewable feedstocks for value-added products. *Bioresource Technology, 337*, 125464.

Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology, 83*(1), 1–11.

Singleton, P., & Sainsbury, D. (2001). *Dictionary of Microbiology and Molecular Biology*. Wiley-Blackwell.

TeBeest, D. O., Caesar, A., & Auld, B. (1992). Virulence of fungal pathogens in weed control. *Biocontrol Science and Technology, 2*(3), 257–269.

TeBeest, D. O., Yang, X. B., & Cisar, C. R. (1992). The status of biological control of weeds with fungal pathogens. *Annual Review of Phytopathology, 30*(1), 637–657.

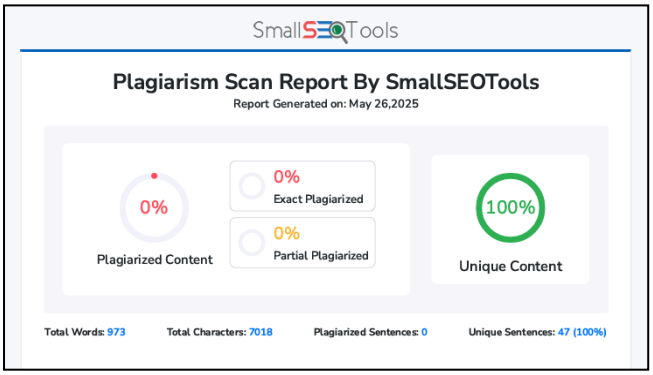
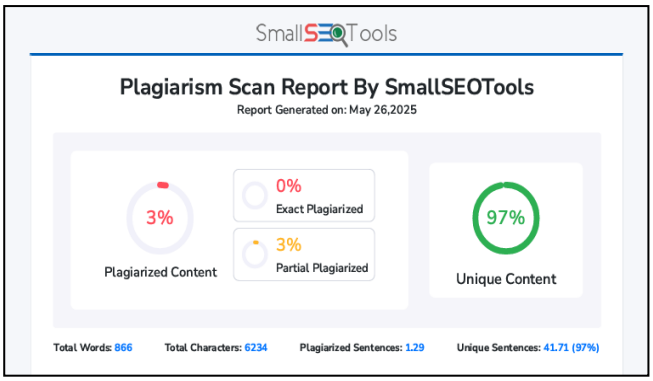
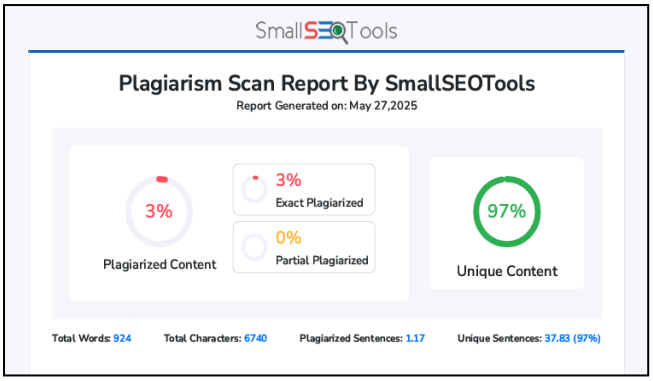
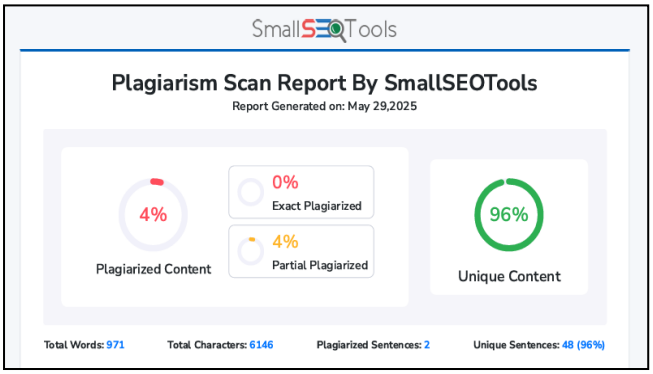
Villamagna, A. M., & Murphy, B. R. (2010). Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): A review. *Freshwater Biology, 55*(2), 282–298.

Vurro, M., Boari, A., Pilgeram, A., & Sands, D. C. (2001). Exogenous amino acids affect the phytotoxic activity of *Fusarium*-derived bioherbicides. *Weed Technology, 15*(4), 835–845.

Wapshere, A. J. (1974). A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology, 77*(2), 201–211.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (pp. 315–322). Academic Press.

Zhao, Z., Liu, H., Wang, C., & Xu, J. R. (2019). Comparative analysis of fungal genomes reveals dif
Gopal, B. (1987). *Water Hyacinth*. Elsevier Science.

.

PLAGIARISM REPORT